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ANNUAL REPORT FY 1977

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM-
Division of Clinical and Behavioral Research
Division of Biological and Biochemical Research
Division of Special Mental Health Research
NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1976 - September 30, 1977

VOLUME II - INDIVIDUAL PROJECT REPORTS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00092-03 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Central Amines and Psychiatric Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A three-year collaboration between the National Institute of Mental Health (NIMH) and the National Naval Medical Center (NNMC) has resulted in the study of central amine metabolites in the cerebrospinal fluid (CSF) of about 120 psychiatric patients, largely personality disorders and alcoholics. Results to date regarding significant differences of personality disorders from normals are inconclusive. Alcoholics appear to have an increased 5-hydroxyindoleacetic acid (5HIAA) in relation to acute intoxication. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE). All results are preliminary and data is being analyzed for subsequent publications. These patients have also been used as control data for other psychiatric diagnostic groups at NIMH.

Project Description:

Objectives: A growing body of evidence obtained in recent years indicates that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5-HT), and gamma-amino butyric acid (GABA) act as central nervous system neurotransmitters and/or modulators of central nervous system transmission. Although considerable indirect pharmacologic evidence has linked functional abnormalities in these amine systems with psychiatric illnesses (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the applicability of these hypotheses to improved diagnosis and treatment of the major psychiatric disorders. Relatively little direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. As well, recent data suggests that affective disorders and psychoses may yield to biological sub-typing which would enable a more effective choice of pharmacological therapy for individual patients. In addition, there is virtually no data on central neurochemical function in the various characterological disorders - a rather striking deficit in our knowledge, considering the evidence suggesting that some character disorders have patterns of family history transmission suggestive of a genetic component. Recent evidence from animals suggesting a close correlation between aggressive behavior and biogenic amines, for example, renders this a particularly interesting area for exploration at this time. Alcoholism is also an area in which neurotransmission needs more study. The purpose of this present project would be to extend the studies of central amine turnover into larger populations of psychiatric patients including manic-depressive psychosis, schizophrenia, various personality disorders, and alcoholism.

Methods Employed: Recently at the NIMH a technique has been developed which allows for the direct estimation of brain amine turnover in psychiatric patients. This method involves the administration of probenecid, a drug which blocks the process by which the amine metabolites are removed from the brain-cerebrospinal fluid (CSF) system into the blood. Following blockade of their removal, the metabolites of the amines accumulate in the spinal fluid at a rate which is in direct proportion to their synthesis or turnover rate in the brain. Experiments with this technique conducted over the last seven years at the Clinical Center of the NIH have led to the following conclusions: (1) experience in over 300 patients has indicated that both the lumbar puncture and the administration of probenecid are safe procedures not associated with significant side effects or toxicity, except for nausea; (2) studies of this technique in conjunction with specific amine precursors and synthesis inhibitors have provided direct evidence that the probenecid induced accumulation of amine metabolites does reflect amine synthesis and turnover rates; (3) application of this technique has been shown to be capable of uncovering differences in central amine function among subgroups of patients with manic-depressive illness and other functional psychotic states. These differences were often not detectable by measurement of amine metabolite baseline levels without probenecid.

Major Findings: About 120 patients have participated in this study to date. Preliminary findings suggest that a group of personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgement, when compared to a mixed control group from NIMH (normals and patients with non-CNS neurological disorders), have similar baseline 5-hydroxyindoleacetic acid (5HIAA) and elevated homovanillic acid (HVA). More of these patients need to be studied. A group of 15 personality disorders have also shown no statistically significant difference in CSF cyclic 3,5-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from groups of depressive, manic, and schizophrenic patients. 3-Methoxy-4-hydroxyphenylglycol (MHPG), vanillylmandelic acid (VMA), dopamine beta-hydroxylase (DBH), GABA, and NE CSF assays have been done on a limited number of patients.

A group of alcoholics, now about 80, have also been studied utilizing the same assays above. It has been found that alcoholics do not differ from personality disorders with respect to HVA. However, early studies do indicate that the mean 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in relation to the length of abstinence to reach a baseline value not differing from the personality disorders. An additional study shows that though HVA values do not change in relationship to the time period post intoxication-withdrawal, they are depressed concomitant with the use of disulfiram (Antabuse), a DBH inhibitor. In addition, disulfiram use correlates with a significant increase in serum NE. Mean serum DBH in alcoholics vs. normal controls was significantly lower, blood pressure was significantly higher, and serum NE was not different. A number of studies involving MAO in the platelets of alcoholics are also underway.

Significance for Mental Health Research: Central cerebral functioning is greatly under-studied in two major groups of psychiatric patients, namely personality disorders and alcoholics. Studies of animal models and hyperactive children suggest a relationship between central neurotransmitter systems and aggressive behavior. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also drug-free personality disorders serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. This project was submitted to the Navy for reapproval in March 1977. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC.

Publications:

Smith CC, Tallman JF, Post RM, van Kammen DP, Jimerson DC, Brown GL, Brooks BR, Bunney WE Jr: An examination of baseline and drug-induced levels of

cyclic nucleotides in the cerebrospinal fluid of control and psychiatric patients. Life Sci Vol. 19, pp 131-136, 1976

Major LF, Ziegler MG, Lake CR, Brown GL: The effects of disulfiram on plasma norepinephrine in male alcoholics. Currents in Alcoholism Frank A. Seixas, M.D., F.A.C.P. (Ed) Vol. 1, 1977

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00126-08 BP

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Family Views of Its Social Environment: Effects on Family Therapy Process

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No work was done on this project during this period since principal investigators had left NIMH.

This project is hereby terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00021-12 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of Sleep

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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CHECK APPROPRIATE BOX(ES)

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental findings over the past decade promise understanding of the basic biochemical process underlying sleep and the increasingly clear demonstrations of severe sleep disturbances associated with psychiatric disorders, as well as the existence of sleep disorders themselves, indicating need for better means of alleviation. To the extent that the process of sleep can be understood, more effective treatment of disorders of sleep will surely follow. In addition to the clinical, biochemical and endocrinological work this laboratory has done in the past, we are beginning to ask fundamental questions about the nature of sleep, using chemical and psychological probes.

These studies contribute to the extensive national and international efforts to understand why we spend one-third of our lives in this state.

PHS 6040

Project Description:

Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent understanding of sleep can be achieved, more effective treatment of its disturbance will surely follow.

Major Findings: Pharmacological

A. Relationship of Cholinergic Activity to REM Narcoleptic Attacks

In order to explore the possible relationship between levels of cholinergic activity and REM narcoleptic attacks, we have begun therapeutic trials of the cholinergic blocking agent, Artane (trihexyphenidyl 2 mg. tid). Results from the first three narcoleptic patients have been encouraging in that all three appeared to show clinical improvement, especially in terms of less frequent attacks of cataplexy. The converse procedure of administering physostigmine (0.5 mg i.v., q 5 minutes to a total dose of 1.5-2.5 mg) in an effort to determine whether increased cholinergic activity induces REM narcoleptic cataplexy did not have any discernible effects in the same three patients. In addition, physostigmine had no apparent effects on the mood of these patients, contrary to recent reports involving psychiatric patients.

B. Time-dependent Effects of Physostigmine on Normal Human Sleep and Arousal

We have recently found that REM sleep was induced by 0.5 mg physostigmine (phy) i.v. 35 minutes after sleep onset, in normal subjects pretreated with methscopolamine (0.5 mg i.m.). We have now extended the study to include other time periods. Thirty-three normal volunteers received 40 physostigmine (phy) and 40 matched saline (sal) infusions. (1) With postinfusion-arousals excluded, infusions given 5 mins after sleep onset reduced time from infusion to onset of REM from 78.9 $\pm$ 14 mins (sal) to 52.4 $\pm$ 10.4 (phy) ($p<0.05$, two-tailed "t" test). (2) Infusions given 35 mins. after sleep onset reduced time from infusion to onset of REM from 65.7 $\pm$ 15.2 (sal) to 11.2 $\pm$ 2.1 (phy) ($p<0.005$). REM was induced more rapidly by physostigmine infusions given 35 mins after than 5 mins after sleep onset ($p<0.01$). (3) For the infusions at REM onset, 5 mins after and 25 mins after REM, the predominant effect was one of arousal. Judging from number and duration of awakenings, the order of intensity of arousal was: REM > 5 mins after > 25 mins after REM. Apart from "true" awakenings, two other types of arousals were seen:

Also occurring EEG/EMG dissociation with alpha EEG, muscle atonia and rapid eye movements resembling sleep paralysis. This was seen in three subjects who were given 1.0 mg dose and was accompanied by early morning

reports resembling sleep paralysis (e.g., "I was awake but couldn't move my arms.") "Alpha REM" characterized by periods of alpha EEG with muscle atonia and without eye movements alternating with conventional REM sleep.

These data suggest that cholinergic mechanisms are involved in initiating REM sleep and arousal. During the first REM period, physostigmine more readily induces REM when given 35 min than when given 5 min after sleep onset, whereas the same dose given at REM onset and during the second REM period produces different intensities of arousal.

Using single bolus infusions of physostigmine administered at different times during the first and second REM periods, we have recently shown that REM sleep and arousal can be induced in a time- and dose-dependent manner (1). In view of the extremely short-lived action of a bolus infusion of physostigmine we have hitherto not been able to determine if the duration of a REM period can be prolonged in addition to triggering its onset.

Seven normal male volunteers (Mean age = 22.8) were studied for three nights (one "adjustment" and two experimental nights). Subjects were pretreated with 0.75 mg IM methscopolamine prior to bedtime; and during sleep they received either placebo or physostigmine 1.0 mg given by a slow IV drip over a period of 60 minutes. The IV infusion began 35 minutes after sleep onset and ended about 95 minutes after sleep onset.

Physostigmine significantly decreased both the latency of the first REM period (Placebo = 108.4 $\pm$ 16.2 mins. (mean $\pm$ S.E.) physo = 61.7 $\pm$ 2.2 mins., $p < .05$ and the duration of the second NREM period (placebo - 110.4 $\pm$ 13.6, physo - 74.4 $\pm$ 6.5, $p < .05$). The duration of individual REM periods were not altered. Although total REM time and REM per cent showed a non-significant increase of physostigmine (RT = 98.2 $\pm$ 6.4 mins., R% = 25 $\pm$ 1 5%) as compared to placebo nights (RT - 80.9 $\pm$ 9.4, R% = 20.1 $\pm$ 1.9%) this can be accounted for by the greater number of REM periods on physostigmine (4.3 $\pm$ 0.3) than on placebo (3.6 $\pm$ 0.2) conditions. Cholinergic mechanisms may modulate the "timing" rather than the "maintenance" of REM.

To examine the psychological process of dreaming in man, seventeen normal volunteers (11M, 6F, mean age = 23) after pretreatment with methscopolamine 0.5 mg IM slept with an IV needle and at either 10 or 35 minutes after sleep onset received one intravenous infusion per night of either placebo or physostigmine 0.5 mg.

During Experiment I subjects were given an IV infusion 10 minutes after sleep onset and were awakened 20 minutes after infusion. Experiment II includes both 7 and 20 minute awakenings; in addition subjects were instructed to go back to sleep after the 20 minutes awakening and were then awakened approximately 7 minutes after they had spontaneously gone into REM sleep. The above study was designed to control for (1) time of infusion, (2) time from infusion to awakening, (3) state of sleep during awakening.

At each awakening a taped interview was conducted. Subjects were questioned waking. After the interview subjects filled out a questionnaire. A dream mentation scale was constructed from the questionnaire ranging from +5 (absolute confidence in presence of dream) to -5 (absence of dreaming).

	Experiment I		Experiment II				
	Awakenings 20 min after infusion		Awakenings 7 min after infusion		Awakenings 20 min after infusion		Spontaneous REM sleep awakenings
	Physo	Placebo	Physo	Placebo	Physo	Placebo	
1) No. of awakenings	10	10	9	9	10	10	10
2) Sleep state during awakenings	non-REM	non-REM	non-REM	non-REM	REM (9) non-REM(1)	non-REM	REM
3) No. positive dream recall (%)	4 (40%)	2 (20%)	4 (44%)	3 (33%)	8 (88%)*	2 (22%)	7 (70%)
4) Dream mentation (mean ± SEM)	not obtained		-0.4	-1.2 ±1.3	+2.3* ±0.9	-2.0 ±0.8	+2.3 ±0.9

* $p < .01$

During Experiment I and the 7 minute post-infusion awakenings of Experiment II neither REM sleep nor dreaming were induced by physostigmine. In the 20 minute post-infusion, physostigmine induced the onset of REM sleep in approximately 13 minutes in all but one subject. Those drug-induced REM episodes were associated with significantly higher incidence of dream recall than placebo.

This study indicates that the physostigmine-induced physiological characteristics of REM sleep are accompanied by dreaming. The dreaming occurring during physostigmine-induced REM periods appears to be similar to that observed during spontaneously occurring REM sleep.

C. Identification of a Stimulatory Cholinergic Mechanism in Sleep-related Growth Hormone Secretion.

We have examined the effects of cholinergic blockade with methscopolamine bromide, 0.5 mg intramuscularly, on sleep-related and insulin-induced growth hormone (GH) and prolactin (PRL) secretion. Thirteen normal young men were studied; eight had sleep studies, and at different times in 3 sleep subjects and in 5 others not studied during sleep, insulin tolerance tests were performed with 0.1 unit/kg insulin. After an adjustment night in the sleep laboratory, saline control and methscopolamine studies were done in a random sequence; study procedures included electroencephalographic, electromyographic, and electro-oculographic recordings, and blood sampling every 20 minutes for hormone radio-immunoassays. On methscopolamine nights, the mean ± SEM overall

GH level of 2.89 ± 0.44 and the mean peak GH level of 11.09 ± 3.11 ng/ml were dramatically reduced to 0.75 ± 0.04 and 1.04 ± 0.25 ng/ml, respectively ($p < 0.0001$ and $p < 0.001$). Despite virtual absence of GH secretion during the night in every study subject, no measured sleep characteristic was significantly affected by methscopolamine, including percentage slow-wave sleep (12.1 ± 2.6% control vs. 10.3 ± 2.5% drug). Sleep PRL concentrations were not changed by methscopolamine. In contrast to the abolition of sleep-related GH secretion, administration of methscopolamine only marginally reduced the GH response to insulin hypoglycemia. Only one of ten time points differed significantly, and the peak concentrations, mean increments, and areas under the curves were not significantly different. We conclude that the burst of GH secretion which normally occurs following sleep onset is primed by a cholinergic mechanism which does not influence slow-wave sleep. Cholinergic mechanisms outside the central nervous system do not appear to play an important role in sleep-related prolactin secretion. The neurotransmitter mechanisms, and presumably the pathways, which subserve sleep-related GH secretion in man appear to be different from those which mediate the GH response to pharmacologic stimuli such as insulin.

D. Effects of L-Histidine on Sleep

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electrographic sleep patterns. L-histidine, was given to three patients with intractable narcolepsy (20 gm per day for 2 weeks), to four normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day for 16 days. No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

E. Differential Effects of D- and L-Amphetamine on the Sleep of Depressed Patients On and Off Lithium Treatment.

Dextro (d-) and levo (l-) amphetamine produced different EEG sleep changes in seven depressed patients. Each patient received placebo or one of the isomers (30 mg base) at 7:25 a.m. in a double blind fashion. The patients were studied under two conditions: without treatment with lithium carbonate and with treatment with lithium carbonate (0.9-2.1 gm/day beginning a minimum of 10 days before study). Both isomers reduced REM sleep and the proportion of total sleep spent in REM (the REM%). No REM rebound was observed on the night following REM suppression. Only d-amphetamine delayed sleep onset and reduced Total sleep time, NREM sleep time, and sleep efficiency. The same changes were observed with and without lithium carbonate treatment.

F. A Dose-response Study of the Acute Effects of Ethanol on the Sleep of Rats.

Although there is no shortage of studies on the effects of ethanol on sleep of animals, there is little dose-response data available. In order to examine this relationship more carefully, we have administered increasing doses of ethanol (1.1, 1.5, 2.0, 2.5 g/kg) and equivolume saline intraperitoneally to five groups of six rats each. Injections were given five minutes before the beginning of seven-hour recordings, from 9:00 a.m. to 4:00 p.m. The percent of REM decreased and the percent of slow-wave sleep increased. By dividing the recording into sequential 3.5 hour periods, it became clear that this effect occurred most strongly in the first 3.5 hours of sleep. Although the number of awakenings was not changed over the recording as a whole, they were significantly increased in the second half of the recording. This may be a reflection of greatly decreased blood alcohol levels. (A dose of 2.5 g/kg is completely metabolized in about six hours in the rat.) It is of interest that even the highest doses of ethanol did not produce prolongation of total sleep time in either the whole recording or the first or second halves.

G. Hypnotics and Ethanol: Residual EEG Effects.

The literature is divided between those who have found a possible synergistic interaction of ethanol and benzodiazepines and those who have not. We have examined this relationship by recording 12-minute waking clinical EEG's in 47 normal young men who received 0.25 mg triazolam, 30 mg flurazepam or placebo, alone or in combination with 0.8 g/kg ethanol. EEG's were performed as a baseline (3:00 p.m.), 50 minutes after receiving drug or drug placebo (5:50 p.m.), 50 minutes after receiving ethanol or ethanol placebo (7:05 p.m.) and 16 hours after having received the drug (9:00 a.m.). Criteria for the presence of drug effects were:

1. Increase in amount and amplitude of sinusoidal rhythmic beta activity.
2. Lower amplitude of background activity.
3. Decrease in amount of alpha activity
4. Presence of "well-modulated" alpha.
5. Presence of "drug spindles."

Eighty-five percent of the baseline records were correctly identified as having no drug effect. Analysis of the second EEG (50 minutes after drug) and third EEG (50 minutes after ethanol) showed that our interpretations had a significant relationship to whether or not the subject had received the active drug ($p<0.02$ and $p<0.05$ respectively). These findings lead us to believe that this method for determining the presence of drug effects is valid.

Analysis of the fourth EEG--16 hours after drug administration--showed no overall difference in incidence of EEG drug effects among the various combinations of drugs and ethanol. Neither drug alone was different from placebo, nor was either drug alone different from itself plus ethanol.

Flurazepam plus alcohol produced drug effects in 86% of the records, compared to 22% in the placebo group ($p<0.019$; Fisher Exact Probability Test). The difference between incidence of drug effects following trizolam plus ethanol (56%) and placebo (22%) did not reach statistical significance. There was no difference in frequency of effects between flurazepam plus ethanol and triazolam plus ethanol.

These data lead us to believe that ethanol produces a small but significant increase in the duration of EEG effects of flurazepam. Flurazepam alone is known to remain active for some time (the major metabolite may have a half-life of 50-100 hours). Thus, repeated use of the drug might produce cumulative effects which may possibly be increased by ethanol.

H. Serotonin: Relationship to Narcolepsy.

Since serotonin has been implicated in the induction and maintenance of sleep, we administered para-chlorophenylalanine (PCPA 3 gm/day for up to 4 weeks) to 3 patients with narcolepsy. No consistent change in sleep or clinical symptomatology was observed in the 3 patients as a group. One patient, in contrast to our previous experience with PCPA in patients with carcinoid syndrome, actually showed a significant increase in REM while on PCPA, while the other patients showed no decrease in REM. These results suggest (a) that narcolepsy is not related to an abnormality of serotonin and (b) that narcoleptics respond differently to PCPA than carcinoid patients.

Major Findings: Clinical Disorders

A. EEG Sleep Stages Compared in Normals, Insomniacs, and Depressed Patients.

All-night EEG sleep characteristics of normals, insomniacs, and hospitalized depressed Bipolar and Unipolar Patients were compared. Following an adaptation night, subjects were recorded for three to six nights in the same laboratory setting. All had been drug free for a minimum of two weeks. The depressed patients met criterion of primary affective disorder and were hospitalized because of significant clinical depression at the time of the study. The insomniac patients, who have been described previously, gave a history of significant, prolonged insomnia, did not snore, and were free of significant psychiatric signs, symptoms, or history.

The means were:

	Normals	Insomniacs	Unipolars	Bipolars	ANOVA
Number	41	18	33	23	
Mean Age	41	46	51	41	
Total sleep, min	378	339	286	328	$p<.001$
REM, min	90	86	78	88	
REM Latency, min	78	66	46	46	$p<.001$
Delta, min	24	9	7	16	$p<.005$
Inter. Awake, min	29	40	54	31	$p<.05$
Early Morning Awake	5	9	43	33	$p<.001$

After entering sleep variables into a step-wise, multiple discriminant analysis, 73% of the subjects were correctly classified into their predicted diagnostic group:

	Predicted Group Membership (Percentage)			
Actual group	Normals	Unipolars	Bipolars	Insomniacs
Normals	100.0	0	0	0
Unipolars	27.3	60.6	12.1	0
Bipolars	13.0	34.8	52.2	0
Insomniacs	22.2	0	16.7	61.1

These results indicate that all disorders of sleep are "not the same," and that all night EEG sleep studies provide reasonably successful classification of depressed Bipolar, depressed Unipolar, and Insomniac patients.

B. The "Switch Process" in Manic-depressive Illness: Circadian Variation in Frequency of Switches and Changes in Total Sleep Time.

A "switch" was operationally defined as a rapid change in mood (change of three or more points on a 15-point scale) occurring in less than 24 hours. Out of 75 bipolar manic-depressive patients, 35 showed 70 switches from depression to mania (D→M) and 37 switches from mania to depression (M→D). Next, they were subdivided according to time of occurrence (i.e., which nursing duty shift they occurred in; 11 p.m. to 7 a.m., 7 a.m. to 3 p.m. or 3 p.m. to 11 p.m.). Average sleep time as estimated by nursing staff for five days before (Day -5 to -1) and five days after the day of switch (Day 0 to +4) was calculated. The day of switch was designated as Day 0.

Both D→M and M→D switches occur predominantly in the morning (7 a.m. - 3 p.m.). Among the D→M group, patients who switch at night (11 p.m.-7 a.m.) show significantly greater sleep loss for the next four days (Day 0 to +4), than if they switch in the morning (7 a.m.-3 p.m.) ($p<0.01$, Newmann Keuls test). The evening shift does not differ from the other times. The M→D switches, however, fail to show a similar differentiation between the three time shifts with regard to total sleep time. These data indicate that time of switch may predict subsequent sleep changes in D→M switches.

In an unmedicated female bipolar patient fifty-eight all-night polygraphic sleep recordings were obtained during eight switches into and out of mania. While depressed she was hypersomniac and exhibited elevated REM sleep time and short REM latency. On four nights, she apparently switched into mania while asleep. The last recorded sleep stage in each case was REM sleep.

We have analyzed 144 switches (80 switches into mania and 64 out of mania) in another bipolar patient with regular 48-hour manic-depressive cycles. Mrs. J was 41 when she was first admitted to the NIH Clinical Center in 1962 and was hospitalized several times over the course of two years. During her inpatient stay she was studied intensively; the nursing research team rated her every two hours on a 15 point mania scale. Checks were made every fifteen minutes during the night to determine whether

she was asleep. We defined a "switch" into or out of mania as a change of manic ratings of at least three points.

Next we tried to localize the specific two-hour time period in which a switch took place using nursing ratings. Switches into mania showed a sharp tendency to occur between 8 p.m. and 8 a.m. with peak incidence between 2 a.m. and 4 a.m. Similarly, switches out of mania occurred significantly more often between 4 a.m. and 6 a.m. than during other time periods. The frequency of distribution of these switches into and out of mania was significantly different from an expected random distribution (2XR chi square analysis with df=11). Switches during sleep: Among the 80 switches into mania 37 occurred out of sleep (41%). Among the 64 switches out of mania, 27 were associated with sleep (42%).

C. 5-Hydroxytryptophan and Schizophrenia

5-Hydroxytryptophan was studied at doses of 200-6000 mg/24 hr with MK486 in 18 chronic schizophrenic patients. EEG sleep studies revealed: (a) REM sleep was reduced at all doses; no rebound was found with 5HTP withdrawal; (b) REM latency was increased at all doses; and (c) NREM sleep was increased at low doses, decreased at high doses, and decreased still further during withdrawal.

D. Schizophrenia

Eight actively ill schizophrenics and eight nonpsychotic controls were deprived of REM sleep by the awakening method for two nights. Sleep patterns during five post-deprivation nights were analyzed by a variety of multivariate techniques.

Data suggest that actively ill schizophrenics are less likely than control psychiatric patients to exhibit a normal REM rebound. They require fewer awakenings than controls to achieve REM deprivation. They show little or no change in REM time or REM% during recovery as compared with baseline, and, compared with controls, have significantly less REM time, REM%, and change in REM time and REM% on early post-deprivation nights.

The two patient groups also differed in their pattern of stages III and IV during recovery. Considerable overlap existed in REM compensation between actively ill schizophrenics and controls. Additional information suggests that REM compensation may be related to Rod and Frame testing: the more field independent a subject is, the better REM compensator he is.

E. Absence of Fast Ocular Activity During REM Sleep in Familial Cerebellar Degeneration

The major sleep abnormality in a family with a hereditary cerebeller degeneration was an absolute loss of fast ocular activity during REM. The more affected the individual was with the disorder, the less likely he or she was to display fast ocular movements during REM sleep.

In the absence of neuropathological evidence, it is impossible to precisely characterize the neurologic deficit in this disorder, and, thus, perhaps provide a neuroanatomical basis for the REM's of REM sleep.

Clinically, the disease appears to involve the cerebellum predominately. It is known, however, from animal studies that cerebellectomy does not affect the REM's of REM sleep.

Although vertigo was not a prominent symptom in any of these patients, the electronystagmography results suggest a central vestibular lesion in three patients. While this data is only suggestive, it is consistent with the hypothesis put forward by Pompeiano that REM's depend upon the integrity of the medial and descending vestibular nuclei. The intellectual relevance to the absence of REM's since Feinberg has reported diminished REM's in the senile.

F. The Relationship between Changes in REM Sleep and Clinical Improvement in Depressed Patients Treated with Amiptriptyline

EEG sleep recordings were obtained on consecutive nights from six hospitalized depressed patients before, during, and after treatment with amitriptyline. Amitriptyline significantly reduced time spent in Rapid Eye Movement (REM) Sleep and prolonged the REM Latency throughout the treatment period. Three patients who improved during treatment had a REM rebound when amitriptyline was discontinued, whereas three patients who did not improve showed no REM rebound.

Major Findings: Normal Studies

A. Effects of Sleep Stage and Stimulus Intensity on Auditory Evoked Responses

The purpose of this study was to evaluate the effect of sleep stage and stimulus intensity on the auditory evoked response (AER). Nine normal volunteers (age 18-22; 6M, 3F) slept in the sleep lab for three nights each; the first night was adaptation and was not included in any calculations. Four intensities of clicks (50, 60, 70 and 80 dB) were generated by the LINC computer in pseudo random order. EEG was sampled at 250 Hz for 1 second and AER was computed on line for each intensity for each minute of the night. Each minute was visually scored for sleep stage. AER from the minutes of each sleep stage were then averaged. AER amplitude tended to increase little from 50 to 80 dB in waking subjects but increased markedly in sleeping subjects in stages 3 and 4 ($p<0.05$, 2-way analysis of variance). Rapid eye movement (REM) and stage I sleep had small amplitude AER's in comparison with other sleep stages.

Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during the experimental night ($r=-.76$, $p<0.05$). The loss of inhibitory mechanisms during stages 3 and 4 is well known. It is therefore not surprising to see the disappearance of the paradoxical AER amplitude reduction at high intensities and a more linear EP amplitude/stimulus intensity function; stage 1, 2 and REM

sleep resembled the waking state. Of interest was the finding that waking AER amplitude/stimulus intensity functions predicted sleep duration in this noisy environment. This suggests that individual differences in sleep duration may be related to differences in inhibition of sensory processing.

Major Findings; Animal Behavior

A. Deprivation of Rapid Eye Movement Sleep and Nesting Behavior in Rats

The effects of REM deprivation for four days on nesting and locomotor activities were studied in male rats. Four groups of animals were tested. (a) Experimental animals (SP), deprived of REM on a small (6.5 cm) pot; (b) Large pot (LP) control, maintained on large (12.5 cm) pots; (c) stress group, forced swimming for 1 hr/day; (d) normal controls. The results indicate, as predicted, increased utilization of nesting material by the experimental (SP) group in the post-REM deprivation period as compared with normal controls (464 cm for SP, 94 for normals, $p<.05$). Nevertheless, the SP group did not differ significantly from LP (356 cm) or stress (133 cm). Since LP does suffer some REM deprivation, though less than SP, the results suggest that increasing levels of REM deprivation may increase a drive-related behavior in rats.

B. EEG Documentation of a Method for Sleep Depriving Rats

A variety of methods have been employed to produce total sleep deprivation in small animals. These include continuous handling, the use of treadmills or small platforms surrounded by water and administration of amphetamines or electric shocks. Unfortunately, these techniques are largely without support of EEG data. Most also have clear disadvantages. The use of small platforms surrounded by water is an outgrowth of a procedure for REM sleep deprivation; when used for purposes of total sleep deprivation it actually may cause a profound suppression of REM sleep with a smaller decrease in NREM sleep, which still occurs. Other approaches, such as continuous handling of the animals, require enormous amounts of labor. There is a need, then, for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. In this abstract we shall describe such a technique, which consists of keeping rats awake by maintaining them in slowly rotating cylinders.

Rats were placed in two Nalgene cylinders (68.6 x 35.6 cm), each of which is divided into six compartments. An electric motor causes them to rotate at 3 RPM. A strip of tubing on the floor of each compartment provides an obstruction over which rats must walk, so that they cannot slide along.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the EEG and EMG of four rats in the moving cylinders for 24 hours and compared them to seven animals in a stationary cage. The moving cylinder reduced sleeping time from 47.0% of the 24-hour period to only 3.8% ($p<0.001$). The small amount of sleep which

did occur was made up of both REM and NREM sleep. The percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect on one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

We have presented a method of reducing total sleep to less than a tenth of normal for prolonged periods of time. Unlike the modified use of the platform surrounded by water, it does not totally suppress one sleep state (REM) while only partially suppressing another (NREM sleep). A much larger number of rats (12) can be studied at one time than in previous procedures. It is anticipated that this will be useful in observing the effects of sleep deprivation in studies dealing with memory, neurochemistry, and possible circulating sleep factors.

EEG ANALYSIS OF RATS IN SLEEP-DEPRIVATION APPARATUS FOR 24 HOURS*

	$\frac{\text{TOTAL SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$	$\frac{\text{REM SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$	$\frac{\text{NON-REM SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$
Control (n=7)	47.0 ± 3.8	7.4 ± 0.5	39.6 ± 3.7
Experimental Group (n=4)	3.8 ± 1.9	0.2 ± 0.0	3.6 ± 1.9
Significance**	$p < 0.001$	$p < 0.001$	$p < 0.001$

* Values represent means ± SEM
** Two-tail t-test

C. Reduction of Brain Serotonin and REM Sleep by Acute Administration of a Tryptophan-free Amino Acid Diet

A variety of studies have suggested that the serotonergic system plays an important role in the regulation of sleep. In order to clarify this role, we have performed sleep studies on rats in whom brain serotonin was decreased by a new technique-the acute administration of a tryptophan-free amino acid diet.

Prior to the sleep study we confirmed the efficacy of this method in a biochemical study on 24 rats. They were trained for a 20-day period to eat their entire daily food supply (an amino acid diet) in 2 hours. On the twenty-first day they were divided into two groups who received the same diet or one of similar composition but lacking tryptophan. Animals from each group were then decapitated at intervals over the next 24 hours. At the end of the first two-hour feeding, serotonin and 5-HIAA concentrations had dropped significantly. They continued to decrease until they reached a nadir eight hours after the start of feeding, at which time serotonin and 5-HIAA levels were 30 and 41 percent of control values, respectively. At 24 hours they had started to increase but were still significantly less than control values.

Sleep studies were performed on five rats in a paired-data, crossover design. As can be seen on the Table, percentage REM sleep was decreased by one-third ($p<.05$) and there was a small but signinificant increase in nonREM sleep. Total sleep time was unchanged.

Our observation that an acute reduction in brain serotonin failed to produce decreases in nonREM sleep is in agreement with the results of two studies each in rats and humans. These data seem inconsistent with the hypothesis that the serotonergic system plays an important role in the production of nonREM sleep.

	Control Diet	Tryptophan-Free Diet	Statistical Significance
Total sleep	519.6±25.7*	529.8±27.0	NS
Sleep latency	45.5±10.6*	35.8±10.6	NS
Intermittent waking (IW/TRP)	33.9± 3.0	30.8± 2.2	NS
nonREM sleep (NR/TST)	87:6± 2.4	91.4± 2.3	$p<0.05$
REM sleep (REM/TST)	12.3± 2.4	8.5± 2.2	$p<0.05$

Values represent mean number of 30 sec. epochs ± SEM.

Effects of tryptophan-free diet on brain serotonin and 5HIAA

Hours after food presentation	Control diet Serotonin	5HIAA	Tryptophan-free diet Serotonin	5HIAA
2	1.43±0.04	0.52±0.06	0.80±0.02***	0.38±0.02**
4			0.62±0.01***	0.27±0.02***
8			0.44±0.02***	0.21±0.01***
24	1.52±0.02	0.56±0.04	0.65±0.02***	0.41±0.04*

Each value represents the mean from four rats ± S.E.M., in units of ug/g brain tissue.

* $p<0.05$ by t-test when compared to mean of combined control values.
** $p<0.01$
*** $p<0.001$

Significance to Mental Health Research:

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of clinical disorders, biochemistry and sleep.

Publications:

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Wyatt, R.J. and Gillin, J.C.: Biochemistry of Human Sleep. In: Williams, R.L. and Karacan, I. (Eds): Pharmacology of Sleep. New York, John Wiley and Sons, 1976, pp. 239-279.

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Gillin, J.C., Wyatt, R.J., Fram, D. and Snyder, F.: The relationship between changes in REM sleep and clinical improvement in depressed patients treated with amiptriptyline. Psychopharmacologia. (In press).

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Mendelson, W.B., Jacobs, L.S., Sitaram, N., Wyatt, R.J. and Gillin, J.C.: Identification of a stimulatory cholinergic mechanism in sleep-related growth hormone storage. J. Clin. Invest. (In press)

Gillin, J.C., vanKammen, D.P., Post, R.M. and Bunney, Wm. E., Jr.: Effects of prolonged administration of pimozide on sleep-EEG patterns in psychiatric patients. Psychopharmacol. Commun. (In press)

Gillin, J.C., Sitaram, N., Mendelson, W.B., Buchsbaum, M.S., Moore, A.M., Jacobs, L.S. and Wyatt, R.J.: Cholinergic modulation of sleep growth hormone, and analgesia in normal volunteers. In: Jenden, D. and Usdin, E. (Eds.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press. (In press)

Mendelson, W.B., Moja, E.A., Gillin, J.C., and Wyatt, R.J.: Reduction of brain serotonin and REM sleep by acute administration of a tryptophan-free amino acid diet. Sleep Res. 6. (In press)

Mendelson, W.B., Stefurak, S.J., Stefurak, M.L.: EEG documentation of a method of sleep depriving rats. Sleep Res. 6. (In press)

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00098-03 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of Children and Grandchildren of Patients with Depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.4	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, and to identify biological markers of a genetically-transmitted predisposition to affective disorders, and to study the interaction between genetic predisposition and environmental factors in children with affective disorders. Our present project is a refined replication of our pilot project reported last year. The major changes involve the inclusion of two control groups as well as a blind interviewer and rater.

Project Description:

Objectives: (1) to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, (2) to identify biological markers of a genetically-transmitted predisposition to affective disorders, and (3) to study the interaction between genetic predisposition and environmental factors in children with affective disorders.

Methods Employed: We are studying three groups of children: (1) 50 children of parents hospitalized at NIMH with a primary affective illness, (2) 25 children of patients hospitalized at NIH because of a physical illness and screened for any affective disorder, and (3) 25 children of "normal" parents with no known physical or mental disorder. All children are seen twice, four months apart. After the second interview, blood is drawn on the 50 children in group 1 for determination of genetic markers as well as blood serotonin levels (In cooperation with Drs. Elliot Gershon and Dennis Murphy).

A social worker obtains at each visit a social history from the parents and administers the Achenbach Scale for childhood psychopathology.

At each visit, the child has a psychiatric interview. The interview is conducted by a blind interviewer and rater, while another investigator observes through a one-way mirror. The interviews are structured, and follow a standard inventory of topics, aimed at eliciting various types of psychopathology such as affective disorders, anxiety reaction, childhood psychosis, delinquency and minimal brain dysfunction. A portion of the interview is aimed at eliciting fantasy material by the use of free drawings, story telling, and free reporting of dreams, T.V. stories, and movies. Other methods used in child psychiatry such as the three wishes, The Despert fables are also employed. Following the interview, the children are rated on the CPRS scale of the ECDEU as well as on a global rating scale for affective disorders, constructed for this purpose and used by the investigators for many years. All the above mentioned procedures pose no hazards for the children.

Major Findings: In our pilot study of the 30 children, seven fell in the severe range and nine in the moderate range on a depression score devised by totalling the affecting rating items in the various scales we used. None of the children were hypomanic or psychotic and there was no correlation between the depressive symptoms and the sex and age of the children. In our current project, we have seen so far 34 subjects as well as their parents.

Significance to Biomedical Research and the Program of the Institute: There is much interest in determining whether affective disorders can be rated to predict biochemical and other physiological measures which may be genetically determined. Also, there is a considerable interest presently

in the nature and prognosis of affective disorders in children and their relationship with affective disorders in adults. It is to be hoped that our present study will help to claify some of the issues mentioned above.

In addition to the potential significance of clarification of the problem of affective disorders in children, this study has the potential of developing preventive measures in children and perhaps (psychotherapeutic and pharmacological) measures to reduce and forestall, or reduce the frequency of, affective disorders in adults.

Proposed Course of Project: (A) We plan to continue our on-going project until all the 100 children have been seen. Following this phase of study, the analysis of all the available data will be done. (B) 1) We plan to continue to follow these patients twice a year for as long as possible. At each follow-up visit, there will be a history given by the parents, using the Achenbach Rating Scale, a psychiatric interview and rating of the child. 2) The parents of all the children will be seen in a conference and the findings will be discussed. When significant psychopathology is found, the family will be counselled about securing psychiatric help for the child. (C) We plan a pharmacological study in which depressed children of Lithium responders will be given Lithium and placebo in a crossover design. Dr. John Bartko serves as a consultant in the design of this project.

Publications: A paper describing our preliminary findings, "Offspring of Patients with Affective Disorders", was read at the Annual Meeting of the American Psychiatric Association in May 1976 and is presently submitted for publication in the Archives of General Psychiatry. Drs. Cytryn and McKnew have recently completed a chapter, "Affective Disorders of Childhood", to appear in Basic Handbook of Child Psychiatry edited by Jos. Noshpitz and published by Basic Books.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00100-02 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Aspects in Childhood and Adolescent Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.05	2.55	2.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An inpatient program with selected overnight stays, for childhood and adolescent neuropsychiatric disorders has been initiated. The condition currently under study are hyperactivity and Gilles de la Tourette syndrome. Pharmacological compounds under study in these disorders include methylphenidate, amphetamine, piribedil, clozapine, and haldol. Pharmacokinetic studies with behavioral correlations are on-going. Central neurotransmitters and nucleotides, and their metabolites are being studied in serum and urine. Early unpublished findings indicate that the amphetamine half-life in children is about 1/3 that of adults. Behavior and motor activity response do not correlate with serum amphetamine levels. These responses occur at or before the peak blood level is reached.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this program are broad. In the most basic sense, the objective is to gain new knowledge of the central nervous system functioning of children and adolescents with especial reference to maturational changes. Compared to the neurobiological knowledge that has been gathered related to adult neuropsychiatry, much less has been gathered in relation to neuropsychiatric disorders in children. An integration of this developing neurobiological knowledge and childhood neuropsychiatry is a pressing need.

Several principles are important to keep in mind in the study of children versus adults: 1) morphological brain maturation is age related, i.e., the reticular activating system, of considerable importance in psychobiological studies, is not fully myelinated until ages 10 to 20; 2) children may have more rapid and dramatic pharmacological responses, both therapeutic and toxic; 3) no clear history of pre-adolescent "addiction" to compounds, i.e., amphetamine, which can effect unequivocal addiction in adolescents; 4) most behavioral symptoms in children that show pharmacological responsivity have more of an apparent organic contribution than those of adults, i.e., motor hyperactivity, enuresis, poor attention span in children versus complex emotional and cognitive aberrations in adults; 5) children have a smaller repertoire of "past experience" than adults which may be related to the dearth of well-formed delusions in children versus those seen in adults. Young children's dreams are also much less complex than those seen in older children or adults; 6) clinical symptomatology is more variable and fluctuating in children than usually seen in adults, i.e., rating scales for endogenous depression in adults are more easily constructed to measure a "constellation" than is the case for childhood depression.

An objective in support of the above is to elucidate the mechanisms of actions of pharmacological compounds in children and utilize new compounds when appropriate. In addition, a number of studies are being undertaken to elucidate cerebral metabolism in specific disorders and possible protein defects in those disorders.

Methods Employed: An inpatient program for children, and adolescents, involving selected over-night stays, has been established on the 2-West Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a more closely defined population of hyperactive children. In addition, patients with Gilles de la Tourette's syndrome are also being studied. There are future possibilities for studying children with autism and various metabolic defects in conjunction with other Institutes.

All children are thoroughly evaluated by pediatric, medical, neurological, and psychiatric examinations with all routine and other indicated procedures and laboratory studies. Children are also administered various psychologi-

cal and neuropsychological tests and receive a psycholinguistic examination in a collaboration with NINCD. Neurological examinations are scored carefully according to a rating scale (PANESS).

The problem of a reliable diagnostic and nosological system in child psychiatry is of paramount importance. A parents' questionnaire developed by Achenbach which divides children into nosological groupings by factor analysis is being scored blindly on all admissions and will be compared to those diagnoses ascertained in the usual clinical way and by research rating instruments specific for a disorder (as hyperactivity).

Pharmacological compounds, both standard and those previously unused in children are being studied. Serial plasma pharmacokinetic data are being generated for methylphenidate and amphetamine. These data are studied in conjunction with motor activity and behavioral changes, the methodology for which is described below. Piribedil, a specific dopamine (DA) agonist, is being given in an early Phase II trial in hyperactive children and clozapine, a neuroleptic, is likewise being given in Gilles de la Tourette syndrome.

Behavioral changes in hyperactive children are measured pre- and during, via Connors Teachers Rating Scale. The abbreviated 10-item scale is scored daily by a teacher in a classroom that is an integral part of the study program. On days of serial plasma amphetamine determinations, these behavioral changes are measured hourly.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. The unit contains a memory bank that stores this data for 40 hours. At any time the instrument can be read into a computer for a print-out in numerical form or in graph-form.

Amphetamine is being measured by a radio-immunoassay. Biochemical studies include 24-hour urine collection to study homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). These determinations are divided into day and night collections and will be correlated with drug changes, diet changes, and motor activity changes. Serum half-lives of pharmacological Compounds are being ascertained. Serum norepinephrine (NE) changes as they relate to serum amphetamine levels is being studied.

Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Average Evoked Response (AER) studies are being conducted as they relate to hyperactive children in drug-free and treated conditions.

Major Findings: The evaluations of data regarding diagnoses and various psychological and behavioral findings are preliminary.

Data from psycholinguistic evaluations are being prepared for publication.

Serial serum pharmacokinetic data indicates that children reach a peak serum level of amphetamine with 2-4 hours of an initial dose, however as much as 70-80% will remain in the serum at 4-5 hours when behavioral effects have largely dissipated. Apparent elimination half-lives are about 6 hours. In general, socially appropriate behavioral change, increased attention span and concentration ability, and motor activity have a negative correlation with serum blood levels in the first 4 hours of study but no correlation thereafter. Studies evaluating continuous cognitive performance are now under way, but results are preliminary. Publications with more specific details and analyses of the data are being prepared.

Study of urinary metabolites and serum NE are preliminary.

Electroencephalographic and AER studies are also preliminary.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly hyperactive children, have been considerably studied in the last few years, there are still a number of diagnostic and psychopharmacological questions to be answered. The majority of studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards hyperactive children, Gilles de la Tourette's, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and amphetamine give positive responses in 80% of properly diagnosed hyperactive children, and, likewise, haldol gives a high frequency of positive response in Gilles de la Tourette syndrome, the pharmacokinetics and metabolism of these drugs have not been closely studied in children. The correlations between such basic pharmacological knowledge and clinical effects have also been under-studied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in understanding the biological contributions to childhood neuropsychiatry.

Proposed Course of Project: We plan to conduct this program over an indefinite period.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00101-01 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Behavioral and Electroencephalographic Measures of Enuretic Children and Clinical Response to Placebo, Imipramine and Methscopolamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. C. Gillin, M.D.	Research Psychiatrist	BP	NIMH
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	R. J. Wyatt, M.D.	Chief, Lab. of Clin. Psychopharmacol.	SMR	NIMH
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COOPERATING UNITS (if any)
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LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of studies are planned to investigate severe enuresis in males aged 6-12 using electroencephalographic measures during treatment with placebo or active drugs thought to benefit the condition. Half of the subjects are selected to have behavioral disturbance while half have enuresis as a single symptom. The stage of sleep during which enuretic events occurred were recorded as well as other sleep parameters. At the present time 14 children have been studied.

Project Description:

Objectives: The purpose of this first study is to elucidate the mechanism by which imipramine is clinically effective in enuresis. A second purpose is to compare the sleep patterns of behaviorally disturbed and non-behaviorally disturbed children at baseline and during treatment with imipramine, an antidepressant in general use for a variety of childhood disorders. Enuresis has a strong association with childhood psychiatric disorder although it also occurs as an isolated symptom. It is hoped that through the study of the mechanism by which imipramine acts, and of the sleep patterns of these patients, the underlying pathophysiology of the disorder will be better understood.

Methods Employed: Patients were initially selected by telephone screening on the basis of the severity of the enuresis, and whether the child could be clearly identified as belonging in one of the study groups i.e., with associated behavioral disturbance, or with enuresis as an isolated symptom. The Peterson Quay behavior checklist was used for this purpose. On admission to ward 2-West, a medical, neurological and psychiatric examination was carried out. Three nights of baseline sleep recordings were made. Utilizing a structured psychiatric interview for use with children, a diagnostic evaluation is made during the baseline period. Following three nights of baseline sleep recording, the children are given three sequential outpatient trials of medication. The first period is a single blind placebo trial, followed by a double blind cross over trial of methscopolamine (6 mgm hs) or imipramine (75 mgm hs). At the end of each 2 week trial period, sleep recordings were repeated and blood samples obtained for blood level of imipramine and desmethylimipramine.

Major Findings: Fourteen subjects have been studied to date; there have been no dropouts from the study. The most striking finding from baseline evaluation was that enuretic events occur during all sleep stages and not, as had been previously reported, primarily during slow wave sleep. A) No subject reponded to placebo. B) There was a significant difference between the efficacy of the two active drugs; imipramine produced a significant decrease in enuretic episodes while methscopolamine did not. These findings indicate that imipramine action in enuresis is central rather than peripheral, as methscopolamine has only central anticholinergic activity.

Significance to Mental Health Research: As enuresis is strongly associated with other psychiatric disturbance of childhood, a study of the pathophysiology of the condition may shed light on more general physiological disturbance. The comparison between disturbed and non-disturbed enuretic children may differentiate two separation conditions, an "arousal" disorder occurring in Stage III-IV sleep more common in behaviorally normal children and a disorder related to "lighter" sleep stages more common in behaviorally disturbed children. Methscopolamine has peripheral anticholinergic action but does not cross the blood brain barrier. The failure of methscopolamine

to be effective in enuresis supports a central action of imipramine although other peripheral effects (such as noradrenergic potentiation) can not be ruled out. The data from the first study with 14 children appears sufficiently clear. Therefore, the project has been amended and a new study (Project Number 76-M-384-A) will be conducted to further elucidate the mechanism of action of imipramine in this condition (see below).

Proposed Course of Project: The findings of this first study, that methscopolamine is not clinically effective in enuresis, suggests a series of studies to pursue the action of imipramine in this condition. Two studies are planned. The first will be a comparison of desmethylimipramine and imipramine treatments of enuresis. The reason for this comparison is that as DMI is relatively selective in its central effect of blocking the reuptake of norepinephrine while imipramine has equal potency in blocking both norepinephrine and serotonin uptake. DMI has approximately half the central and peripheral anticholinergic potency of imipramine. If DMI and imipramine are equally effective clinically, it is probable that the central therapeutic effect of imipramine is noradrenergic. If imipramine is more effective than serotonergic or cholinergic mechanisms are implicated, a second study is planned to further dissect the enuresis syndrome. This study will investigate the antienuretic effects of Dalmane, a potent inhibitor of slow wave sleep. A leading theory of the cause of enuresis is that it is a disorder of "arousal". If this is correct, Dalmane should be effective in the treatment of enuresis, at least for those individuals whose enuretic episodes occur primarily during stage III-IV sleep. The individual diagnostic profiles of the children will be examined in relation to response to each of the above agents. It is possible that each agent will be useful for a specific type of enuresis and that the syndrome may prove a heterogeneous disorder.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00151-01 BP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Behavioral and Electrophysiological Response of Norm Children to a Single Dose of Dextroamphetamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	T. P. Zahn, Ph.D.	Research Psychologist	LP	NIMH
	M. S. Buchsbaum, M.D.	Chief, Unit on Perceptual & Cognitive Studies	BP	NIMH
	C. L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS
	H. Weingartner, Ph.D.	Research Psychologist,	LP	NIMH
	M. H. Ebert, M.D.	Acting Chief, Sec. on Experimental Therapeutics	LCS	NIMH
	I. J. Kopin, M.D.	Chief, Laboratory of Clin. Science		NIMH
	W. E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology & Psychopathology, NIMH; Laboratory of Clinical Science, NIMH; Section on Communicative Disorders, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	.75	.25

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[X] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the effects of a single dose of dextro amphetamine on normal children and to compare these effects with the known effects of stimulants on hyperactive children. This will provide information about the action of amphetamine in non-fatigued individuals, as well as address the question of whether hyperactive children respond in a way that is different from normal children.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To determine the nature of response of normal children to dextroamphetamine. 2) To correlate physiological, behavioral and biochemical changes following administration of a single dose of the stimulant. 3) To compare these findings to those obtained with a matched group of hyperactive children.

Methods Employed: During the period covered, 15 normal children will be examined at baseline and during a double-blind crossover administation of either placebo or dextroamphetamine (0.5 mg/kg). The study takes three mornings. All subjects are admitted to 2-West. At each session (baseline, drug and placebo) subjects are given a test battery including reaction time and GSR, Evoked Response, Continuous Performance Test, Paired Associated learning and Short Term Memory Tests, Porteus Maze Test, a structured interview for mood and behavior, and tests of linquistic abilities (ITPA). Motor activity is monitored during each test session. Twenty-four hour urine is obtained following drug and placebo sessions.

Major Findings: To date, nine normal and two hyperactive children have been tested. The majority of the normal children have not exhibited any motor activation following active drug. They exhibit an increased reaction time, improved performance on cognitive tasks and decrease in motor restlessness. These changes are similar to those seen in hyperactive children in the present sample and in other studies. The urinary metabolites of amphetamine and catecholamine metabolites have not yet been assayed.

Significance to Biomedical Research and Program of the Institute: There is considerable interest in the action of stimulant drugs because of their known effects increasing the turnover of the CNS transmitters dopamine and norepinephrine. Animal studies have suggested that preweanling mice respond differently to stimulants than do mature animals, and other studies have indicated that "brain damaged" rats (given lead, or 5-OH-DA) respond "paradoxically" to stimulant drug. That is, they are calmed by stimulant only if the drug is preceded by some CWS trauma. The finding that the stimulant drug response of normal children does not differ from that of hyperactive children argues against the suitability of animal models for the study of hyperactivity of childhood and refutes the diagnostic usefulness of a trial of stimulant drug treatment. In addition, the present findings argue against the presence of an alteration of neurotransmitter function as a specific defect in hyperactive children. The cognitive and behavioral effects of dextroamphetamine are striking in the normal sample. The proportion of children showing drowsiness and decreased AER amplitude following stimulant closely resembles the response of normal adults found in other studies. Finally, the results suggest for a central cognitive effect of stimulants not secondary to counteraction of fatigue.

Proposed Course of Project: It is anticipated that the study will be completed during the period covered in this report.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00028-03 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Affect State Dependent Learning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Weingartner, Ph.D. Visiting Scientist BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study and its findings will be incorporated into Project No. Z01 MH 00495-01 LPP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00029-03 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Uncertainty and Organization in Schizophrenic Thinking

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Weingartner, Ph.D. Visiting Scientist BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study and its findings will be incorporated into Project No. Z01 MH 00495-01 LPP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00030-03 BP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Behavioral Mechanisms of State Dependent Learning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Weingartner, Ph.D. Visiting Scientist BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION

NIMH,ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study and its findings will be incorporated into Project No. Z01 MH 00495-01 LPP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00031-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cognitive Changes Associated with Huntington's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Weingartner, Ph.D. Visiting Scientist BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study and its findings will be incorporated into Project No. Z01 MH 00495-01 LPP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00032-03 BP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Amphetamine State Dependent Learning in Depressed Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Weingartner, Ph.D. Visiting Scientist BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study and its findings will be incorporated into Project No. Z01 MH 00495-01 LPP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00034-07 BP

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Psychological and Physiological Correlates of the Average Evoked Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	R. Coppola	Electrical Engineer Unit on Perceptual and Cognitive Studies	BPB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evoked potentials at varying light intensities were examined in paranoid and nonparanoid groups of schizophrenics and in rapidly cycling bipolar affective disorder patients. Early components at 100-140 milliseconds differentiated paranoid from nonparanoids but did not change with mood cycles; late components did vary with affective mood.

Project Description:

Evoked potential research has been given emphasis by the unit because evoked potentials show high heritability and sensitivity to attentional as well as neuropharmacological manipulation -- coupled with the possibility of cortical or subcortical localization, these features make it an attractive technique for biological research in psychiatry.

A patient with unusually regular and rapid switches from mania to depression was studied for 113 consecutive days through five switches. Average evoked responses (AERs) to four intensities of light were recorded from vertex and occipital leads; telemetered activity records and behavioral ratings were also collected. Late AER components (P200) tended to change amplitude synchronously with the switches, vertex P200 amplitude decreased and occipital P200 amplitude increased in mania. Urinary MHPG changes paralleled the changes in P200 amplitude. Early AER components (P100) and especially the amplitude/intensity slope measures for P100 decreased about 8-10 days before a switch from depression to mania. Cross-spectral and linear regression analysis helped confirm these observations. The results, taken together with AER data in recent L-dopa studies, were consistent with catecholamine potentiation prior to the switch process from depression into mania.

Another study represents an attempt to replicate the central results of earlier work on stimulus intensity control. It uses a diverse sample of 40 hospitalized schizophrenic subjects, both chronic and acute. Two measures of stimulus intensity control were used: the Petrie kinesthetic figural after effects procedure (KFA), and the EEG method of average evoked response (AER). Finding the earlier results generally replicated, this study presents a model of the function of stimulus intensity control in schizophrenia, which suggests that acute schizophrenics are particularly vulnerable to being inundated by stimuli, and therefore, that in order to protect themselves, they tend to reduce the perceived intensity of stimuli. Chronic schizophrenics have higher AER amplitudes and reduce less than acutes, suggesting that among chronics, the reducing mechanism either never was present or failed, whereas paranoid schizophrenics tend to be augmenters -- a response style consistent with vigilant scanning. We noted this last replication of our paranoid schizophrenia findings with special interest since we have previously reported low platelet monoamine oxidase to be associated with augmenting and it appears to be paranoid schizophrenics who especially show low platelet MAO levels.

The Unit developed a major review of the psychophysiology of schizophrenia published as a special issue of the Schizophrenia Bulletin. This issue (Volume 3, Number 1) features neurophysiological as well as autonomic measures and emphasizes possible neuropharmacological correlations.

The Unit has also played a key role in developing the new PDP/11 computer facilities for the NIMH Intramural Program. Richard Coppola has had an active part in setting up the new facility and in providing assistance to several of the projects utilizing the computer system. Connection to the DEC system 10 and the 370 system has also been implemented in order to provide further data analysis capability. The new system will also allow for extension to a truly portable facility (under-airline-seat size) utilizing the same basic software and design. This will permit patient testing off the NIH campus at other psychiatric hospitals, in subjects' homes or in foreign field studies.

Publications:

Buchsbaum, M. S. and Henkin, R. I.: Effects of carbohydrate active steroids and ACTH on visual evoked responses in patients with adrenal cortical insufficiency. Neuroendocrinology, 19: 314-322, 1975.

Schooler, C., Buchsbaum, M. S. and Carpenter, W. T.: Evoked response and kinesthetic measures of augmenting/reducing in schizophrenics: Replications and extensions. Journal of Nervous and Mental Disease, 163: 221-232, 1976.

Buchsbaum, M. S., Post, R. M. and Bunney, W. E., Jr.: Average evoked responses in a rapidly cycling manic depressive patient. Biological Psychiatry 12: 83-99, 1977.

Buchsbaum, M. S.: Psychophysiology and schizophrenia. Schizophrenia Bulletin, 3: 7-14, 1977.

Buchsbaum, M. S.: The middle evoked response components and schizophrenia. Schizophrenia Bulletin, 3: 93-104, 1977.

Buchsbaum, M. S.: The average evoked response technique in the differentiation of bipolar, unipolar and schizophrenic disorders. In Akiskal, H. (Ed.). Psychiatric Diagnosis: Exploration of Biological Criteria. New York, Spectrum, (In press).

Post, R. M., Stoddard, F. J., Gillin, J. C., Buchsbaum, M. S., Runkle, D. C., Black, K. E. and Bunney, W. E., Jr.: Alterations in motor activity, sleep and biochemistry in a cycling manic depressive patient. Archives of General Psychiatry, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00035-05 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical and Psychopharmacological Correlates of the Average Evoked Response in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	F. K. Goodwin, M.D.	Chief, Clin. Psychobiology Branch	CPB NIMH
	D. L. Murphy, M.D.	Chief, Clin. Neuropharmacology	CNB NIMH
	R. D. Coursey, Ph.D.	Assoc. Professor, Dept. of Clin. Psychology	U. of Md.

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, DCBR, NIMH
Clinical Neuropharmacology Branch, DCBR, NIMH
Dept. of Clinical Psychology, University of Maryland

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.6	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
A population of individuals potentially at risk for psychiatric disorders was identified by screening 375 college student volunteers for low platelet monoamine oxidase (MAO) activity Levels. The lower and upper 10 percent in MAO activity were interviewed and average evoked response, personality, perceptual and family history data obtained. Low MAO probands reported more frequent psychiatric or psychological counseling and problems with the law. Families of low MAO probands had an eight-fold increase in suicide incidence over those of high MAO probands. Both in these low MAO probands and in NIH psychiatric inpatients the combination of low MAO and average evoked potential augmenting was associated with increased frequency of suicide or suicide attempts. This suggests that reduced MAO levels, reported previously in patients with affective disorders and chronic schizophrenia, may predict a vulnerability to psychiatric disorder.

Project Description:

We previously found that the combined multivariate use of platelet MAO level and AER allowed us to successfully separate the four groups; normal, schizophrenic, bipolar, and unipolar affective illness, with 72% accuracy. However, since hospitalization stress, sensory isolation, dietary changes, etc., might affect both MAO and AER, we planned to locate normal individuals with extreme values of MAO and to study them with AER measures. This then represented a kind of biochemical "high-risk" study for the major psychoses.

The "high-risk" approach to the study of psychiatric disorders attempts to transcend the cause-effect dilemma by focusing on individuals believed vulnerable to such disorders before they become manifest. To select a pool of vulnerable individuals, nearly all high-risk studies have relied on genetic models and have examined the offspring or relatives of a clearly defined patient sample. Biochemical studies of psychiatric disorders, on the other hand, have usually begun with patients in "known" diagnostic groups and searched for abnormalities. The present study integrates the usual biochemical paradigm with the high-risk one to produce a new biochemically-at-risk strategy. Here, the "proband" is established by a biological measure, not a clinical one. The use of this strategy reduces the heterogeneity of the sample; eliminates the hospitalization, labeling and treatment factors that confound other biochemical studies; and rigorously tests the power of the biological factor under investigation.

Blood samples were drawn from 400 college student volunteers. Individuals with platelet MAO levels in the upper and lower 10% were brought to NIMH for four two-hour sessions. MAO levels in these extreme groups were roughly comparable with those obtained previously in patient samples. We obtained AER data, IQ, personality scales, interview material, biographic information, sleep reports, detailed family history information, height and weight, and performance on eye-tracking and psychophysical tasks (rod-and-frame, continuous performance task, channel capacity, Stroop).

More frequent psychiatric counseling, arrests, elevated test scales on psychopathology items, drug abuse and other problems were observed in low MAO probands. Families of low MAO probands showed an eightfold increase in suicide or suicide attempts over families of high MAO probands.

Because of our genetic studies linking AER augmenting to genetic vulnerability to affective illness, we examined the combination of low MAO and augmenting. Low MAO augmenters had significantly higher scores on the MMPI Schizophrenia Scale than low MAO reducers. Among low MAO augmenters, six subjects had relatives who had attempted suicide whereas there were none among low MAO reducers. This finding was reinforced by examining suicide attempts in 79 off medication psychiatric inpatients on whom we had both MAO and AER data. In the patient sample, male individuals with low MAO and AER augmenting, a pattern also previously associated with bipolar affective disorders, showed a significantly increased incidence of suicide attempts in comparison to either non-augmenting low MAO or high MAO patients.

Attentional dysfunction was seen in individuals with both low MAO and relatively low DBH. Family stresses, however, were relatively low and social contact was increased in low-MAO males.

Taken together, the high social contact, exuberant sensation seeking, elevated MMPI and familial history of suicide or suicide attempts seem minimally suggestive of preschizophrenia and more suggestive of affective illness. However, other familial high-risk studies have shown a higher incidence of suicide than schizophrenia in relatives of schizophrenics and the occurrence of manics and sociopaths as well as schizophrenics appearing in the children of schizophrenics.

Our finding of both psychological deficits and increased social functioning in low-MAO and low-MAO/low-DBH nonhospitalized nonpatients suggests that individual differences in these biological indicators previously observed in psychiatric inpatients are not merely artifacts of hospitalization or treatment but are related to psychological variables of possible relevance to the clinical psychiatric syndromes. Direct relationships between specific monoamine enzyme activity levels and specific psychiatric diagnostic categories, however, seem unlikely. A nonspecific psychiatric vulnerability associated with low MAO is more consistent with these studies.

Publications:

Henry, G. M., Buchsbaum, M. S., and Murphy D. L.: Intravenous L-dopa plus carbidopa in depressed patients: Average evoked response, learning and behavioral changes. Psychosom.Med. 38: 95-105, 1976.

Buchsbaum, M. S., Coursey, R. D. and Murphy, D. L. The biochemical high-risk paradigm: Behavioral and familial correlates of low platelet monoamine oxidase activity. Science, 194: 339-341, 1976.

Murphy, D. L., Wright, C., Buchsbaum, M. S., Nichols, A., Costa, J. L. and Wyatt, R. J.: Platelet and plasma amine oxidase activity in 680 normals: Sex and age differences and stability over time. Biochemical Medicine, 26:254-265, 1976.

Murphy, D. L., Belmaker, R. H., Buchsbaum, M. S., Martin, N. F., Ciaranello, R. and Wyatt, R. J.: Biogenic amine-related enzymes and personality variations in normals. Psychological Medicine, 7: 249-157, 1977.

Buchsbaum, M. S., Murphy, D. L., Coursey, R. D., Lake, C. R., and Zeigler, M. G.: Platelet monoamine oxidase, plasma dopamine-beta-hydroxylase and attention in a "biochemical high risk" sample. J. Psychiatric Research (In press).

Buchsbaum, M. S., Haier, R. J. and Murphy, D. L.: Suicide attempts, platelet monoamine oxidase and the average evoked response. Acta Psychiatrica Scandinavica (In press).

Buchsbaum, M. S. and Murphy, D. L.: Peripheral monoamine enzyme indicators of psychological function and psychiatric susceptibilities. In Usdin, E. and Wiener, N. (Eds.): Biochemistry and Function of Monoamine Enzymes. New York, Dekker (In press).

Murphy, D. L. and Buchsbaum, M.S.: Neurotransmitter-related enzymes and psychiatric diagnostic entities. In Spitzer, R. and Klein, D. (Eds.): Current Issues in Psychiatric Diagnosis, New York, Raven Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00036-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Individual Differences in Sleep and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	J. Leckman, M.D.	Resident, Department of Psychiatry	Yale Univ.
	J. C. Gillin, M.D.	Research Psychiatrist	BPB NIMH
	R. M. Post, M.D.	Unit Chief, Section on Psychobiology	BPB NIMH

COOPERATING UNITS (if any)

Department of Psychiatry, Yale University

LAB/BRANCH
Biological Psychiatry Branch

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Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier findings that 1) sleep deprivation might be beneficial in certain depressed patients and 2) that patterns of average evoked response (AER) were related to individual differences in sleep duration suggested the utility of the AER in clinical sleep deprivation studies. AER data were collected on normal controls, patients with affective illness and medication-free narcoleptics as a comparison. Primary affective disorder patients showed an increase in AER augmenting following sleep deprivation not shown by normal controls.

Project Description:

In earler studies, auditory average evoked responses (AERs) to clicks ranging from 50 to 80 dB were studied in nine normal adults while awake and during sleep. AER amplitudes tended to increase little from 50 to 80 dB in waking subjects, but they increased markedly in sleeping subjects during stages 3 and 4. Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during experimental nights with tone stimulation. This finding combined with our studies on AER amplitude/intensity relationships to information processing suggested the utility of this AER technique in clinical sleep deprivation studies. The AER measure might be useful as a predictor of clinical response or an indicator of the extent of effect of the sleep deprivation procedure.

Twenty-two normal volunteers and 16 affectively ill patients (11 bipolar, 5 unipolar) were studied with our visual EP augmenting/reducing procedure. Each subject was tested on the afternoon of three consecutive days -- after normal sleep, after a night of sleep deprivation and following a night of recovery sleep.

Primary affective disorder patients, especially bipolar patients, showed an increase in augmenting following sleep deprivation which was maintained following the "recovery" night. This effect of sleep deprivation was statistically confirmed in the patient group. Normal subjects showed a small decrease in augmenting following sleep deprivation and a return toward normal on the recovery night. This difference between patients and normals was confirmed in an ANOVA. Bipolar patients showed this effect more strongly than unipolar patients but bipolar/unipolar differences could not be confirmed statistically.

Thus, sleep deprivation appeared to have a sensorially activating effect on the visual system, perhaps related to the mood-elevating effects seen with patient ratings. In contrast, normal subjects show minimal effects of sleep deprivation.

Publications:

Gillin, J. C., Sitaram, N., Mendelson, W. B., Buchsbaum, M. S., Moore, A. M., Jacobs, L. S. and Wyatt, R. J.: Cholinergic modulation of sleep, growth hormone and analgesia in normal man. In Jenkin, D. and Usdin, E. (Eds.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z0 MH 00037-05 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetic Factors in Psychiatric Illness and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	E. S. Gershon, M.D.	Chief, Unit on Psychogenetics	BPB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Auditory and visual average evoked responses (AERs) were studied in families of patients with affective illness and in a small sample of twins with affective illness. In patients with primary affective illness, affective disorder probands and their siblings showed similar AER amplitude/intensity slopes, as did probands and their spouses. Within the probands' families, the "well" and "ill" relatives were not significantly different, with both groups showing significant augmenting (high amplitude/intensity slopes) in comparison with normal groups. This suggests that AER stimulus intensity slope measures and affective disorders are not transmitted by a single genetic factor but that AER may be related to increased genetic vulnerability. Review of the heritability of other electrophysiologic variables also suggested that multiple loci and dominance, epistasis and interactions may be important.

Project Description:

The role of heredity in psychiatric illness is supported by the many studies demonstrating excessively high rates of illness in persons with a genetic relationship to known patients. The clinically variable expression of psychiatric disorders and the difficulty in identification of mildly affected relatives point to the need for biological markers for the genetic vulnerability. The heritability of AER measures combined with their applicability to both ill and well relatives makes them a promising approach. Further, differences have been demonstrated between patients with schizophrenia and various types of affective disorders.

A study of augmenting/reducing in 30 patients with affective disorders, normal controls, and their first-degree relatives has been carried out to help assess the relationship between the genetics of affective illness and the AER measure. Patients with primary affective disorders had greater increases in AER amplitude with increasing stimulus intensity (augmenting). Siblings tended to have similar AER amplitude-intensity slopes (intraclass r=0.30) as did patients and their spouses. Within the probands' families, the "well" and "ill" relatives were not significantly different, both groups showing significant augmenting in comparison with normal controls. This suggests that AER stimulus intensity slope measures and affective disorders are not transmitted by a single genetic factor but that AER may be related to increased genetic vulnerability.

The association of augmenting and psychiatric vulnerability was also demonstrated in studies of suicide attempts in low MAO affective patients and specially screened volunteers (see Project 5). Genetic mechanisms will be pursued further in planned studies of the heritability of pharmacological effects of amphetamine in normal volunteers.

Publications:

Gershon, E. S., and Buchsbaum, M. S.: A genetic study of Average evoked response augmenting/reducing in affective disorders. In Shagass, C. (Ed): Psychopathology and Brain Dysfunction. New York, Raven Press, 1977, pp. 279-290.

Buchsbaum, M. S. and Gershon, E. S.: Genetic factors in EEG, sleep and evoked potentials. In Davidson, J., Davidson, R. J. and Schwartz, G. E. (Eds.): Human Consciousness and Its Transformations. New York, Plenum Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z0 MH 00038-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Laterality, Psychopathology and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	D. P. van Kammen, M.D.	Unit Chief, Section on Neuropsychopharmacology	BPB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Offic of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Visual AERs from the left and right temperoparietal and occipital cortex were recorded using visual stimuli presented to the left and right hemiretinal fields and to both fields simultaneously. Subjects included normal controls and patients with acute schizophrenia, affective illness and right and left temporal lobectomies for epilepsy. One patient with partial agenesis of the corpus callosum was also studied. Differences in AER caused by attention were noticed especially in the left temperoparietal cortex when stimuli were presented separately to each field. Schizophrenic and lobectomy groups showed diminished asymmetry and attentional effects, whereas bipolar affective disorders showed generally enhanced attentional effects. Full visual field stimulation also produced attentional asymmetries, currently under study, in schizophrenics.

Project Description:

The left cerebral hemisphere has long been recognized as the major processing area for verbal information and the right hemisphere as the area for certain types of nonverbal spatial information. Recently, several psychophysiologic and neuroanatomic studies have suggested that a lateralized deficit may occur in schizophrenia. By presenting visual stimuli in the right and left visual field and recording EEG from the left and right hemisphere, AER techniques can take advantage of the anatomical arrangement of the human visual system to study abnormalities in lateralization. In normal subjects, AERs are highly asymmetrical and differ markedly with the task assigned the subject. AERs from the left temperoparietal area seem especially sensitive to changes in the attention given to the stimulus by the subject. Since attentional deficits have been one of the most widely postulated psychological deficits in schizophrenia, the application of this AER technique seemed promising. A group of 16 off-medication, acute schizophrenics and comparison groups of 17 affective disorder patients and 30 normal controls have been tested. As a further comparison, testing of 15 patients with right or left temporal lobectomies has been accomplished. A report summarizing these results is in preparation.

This technique required monitoring eye movements, restraining subjects' heads and other technical controls. In an effort to simplify the procedure, bilateral visual stimulation studies have been begun in schizophrenic patients and normals. Visual and auditory evoked potentials (EPs) were recorded from the right and left temperoparietal area in 24 right-handed adults under conditions of selective attention to the visual or auditory stimuli and during the execution of verbal and spatial tasks. Selectively attending to one modality over the other caused an increase in EP amplitude which was greatest for a negative component at 120-140 msec, and greater over the right than left hemisphere for both modalities. The verbal and spatial tasks caused no change in hemispheric asymmetry. The results indicate asymmetric functioning of selective attention mechanisms but do not provide evidence for lateralized cortical arousal by unrelated hemisphere-specific tasks.

Publications:

Buchsbaum, M. S. and Drago, D.: Language and hemispheric specialization in man: Event-related cerebral potentials. In Desmedt, J. E. (Ed.): Progress in Clinical Neurophysiology. Karger, Basel, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00039-03

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Sensory Thresholds and Averaged Evoked Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	W. E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BPB NIMH
	G. C. Davis, M.D.	Clin. Assoc., Unit on Studies of Drug Abuse	BPB NIMH
	F. K. Goodwin, M.D.	Chief, Clin. Psychobiology Branch	CPB NIMH
	R. M. Post, M.D.	Unit Chief, Section on Psychobiology	BPB NIMH
	D. L. Murphy, M.D.	Chief, Clin. Neuropharmacology	CNB NIMH
	R. A. Lavine, Ph.D.	Dept. of Physiology	G.W. Univ.

COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH
Clinical Neuropharmacology Branch, DCBR, NIMH
Dept. of Physiology, George Washington University

LAB/BRANCH
Biological Psychiatry Branch

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Office of the Chief, Unit on Perceptual and Cognitive Studies

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Average evoked responses (AER) to varying intensities of electrical stimulation to the forearm and subjective ratings of these stimuli have been studied in a total of 72 patients with affective disorders and 41 age and sex matched normal volunteers. In 12 patients, urinary 3-methoxy 4-hydroxy phenyl glycol (MHPG) was collected during the stimulation session and during a second control session. Physostigmine analgesia and somatosensory AERs were also studied in normal volunteers.

(Continued)

OTHER: M. Webster Research Assistant LCS NIMH
Section on Psychiatry

Project Description:

In 1974 we found strong support for our concept of sensory augmenting/reducing. Individuals who were relatively pain tolerant as indicated by subjective shock ratings exhibited AER to electrical stimuli which decreased in amplitude with increasing stimulus intensity (reducing). A nonparametric signal detection analysis of subjective shock ratings has increased the precision of our AER-shock-rating relationships. This form of analysis is a powerful tool for analyzing pain experiments. It is currently being used in clinical studies of patients, in combination with AER techniques.

We have previously reported that individual bipolar affective disorder patients showed visual AER which increased more in amplitude with increasing stimulus intensity than those of normals or unipolar patients. If the visual and somatosensory data both reflect the operation of a single central stimulus intensity modulator, then we might expect to see the pattern of great rates of amplitude increase for somatosensory stimuli as well. This was confirmed in our initial data analysis based on AERs of 17 bipolar (depressed) patients. Pain ratings also showed bipolar/unipolar group differences.

Patients with affective disorders (n=47) and age and sex matched normals (n=41) were also studied with a pain rating procedure. The patients, 28 bipolar and 19 unipolar (17 men and 30 women), were psychiatric inpatients studied off medication. The electrical stimuli were single pulses ranging from 1 to 31 ma given with a computer controlled constant current stimulator to the dorsum of the left forearm via a Tursky concentric electrode. Nonparametric analogues of response criterion and sensitivity measures were derived for a distinct/unpleasant detection; false positives and false negatives were also analyzed.

Depressed male bipolar patients showed higher pain criteria (Cx) and significantly lower sensitivity (d) than either normals or female bipolars. Depressed female bipolars were no different from normals or female unipolars. Male unipolar patients showed higher Cx than normal controls.

Depressed bipolar males had significantly higher Cx and lower d' than bipolar females; unipolars showed the same trend but it was not significant. No sex differences were observed in normal controls.

Eight patients were tested while manic or hypomanic (2 males and 6 females). These patients showed significantly lower d'. This effect was due to elevations of both false negative and false positive errors.

Pain differences, as do other biological differences, support the distinction between bipolar and unipolar forms of affective illness. Individual, diagnostic and sex differences in pain response may be important clues to the neurobiology of affective illness.

Eight bipolar and four unipolar patients and 10 age and sex matched normal controls had visual and somatosensory EPs, and did a pain rating procedure. During this session and during a comparison session of quiet relaxation, urinary MHPG excretion was measured. Visual and somatosensory EPs were recorded and analyzed. In this study, despite the small numbers of patients, bipolar, unipolar and normal EPs were significantly different, with bipolars showing marked amplitude increases especially at the highest stimulus intensity. This augmenting effect was seen and statistically confirmed for somatosensory stimuli in a lateral somatosensory area lead (C4) and for visual stimuli over the occipital area (O4).

Fourteen normal volunteers were given 0.5 mg physostigmine or saline placebo i.v. in a randomized double blind trial. Subjects were pretreated with 0.5 mg methscopolamine i.m. on both days. Pain ratings and average evoked responses were obtained to brief electrical stimuli administered to the forearm. Pain response criterion was significantly higher on physostigmine than on placebo. Average evoked response component P100 was significantly smaller on physostigmine than placebo, especially for higher intensity stimuli. These results are consistent with central cholinergic modulation of pain and arousal systems in man.

In the current year, we are further refining our EP/psychophysiological techniques with both methodological studies and pharmacological ones including tests of aspirin and naloxone.

Publications:

Sitaram, N., Buchsbaum, M. S. and Gillin, J. C.: Physostigmine analgesia and somatosensory evoked responses in man. European Journal of Pharmacology, 42: 285-290, 1977.

Buchsbaum, M. S.and Murphy, D. L.: Peripheral monoamine enzyme indicators of psychological function and psychiatric susceptibilities. In Usdin, E. and Wiener, N. (Eds.): Biochemistry and Function of Monoamine Enzymes. New York, Dekker (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00040-02 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Amphetamine Pharmacokinetics and the Average Evoked Response in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	M. H. Ebert, M.D.	Chief, Section on Exper. Therapeutics	LCS NIMH
	G. L. Brown, M.D.	Medical Officer	BPB NIMH
	R. D. Hunt, M.D.	Fellow in Child Psychiatry	UCLA

COOPERATING UNITS (if any)

Laboratory of Clinical Science, DCBR, NIMH
Dept. of Psychiatry, Univ. of California at Los Angeles

LAB/BRANCH
Biological Psychiatry Branch

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Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of amphetamine on the average evoked response (AER) is being studied in hyperactive children. Blood amphetamine levels will be correlated with AER measures in an attempt to understand individual differences in response. The AER is only one phase of a larger project reported elsewhere. In an effort to demonstrate possible perceptual response stereotypy associated with amphetamine, single evoked response trials are being collected for both visual and auditory stimuli, These single trials are then being analyzed for signal/noise ratio and trial-to-trial similarity using newly developed signal processing techniques.

PHS-6040
(Rev. 10-76)

Project No. Z01 MH 00040-02 BP

Project Description:

Previous research has demonstrated changes in average evoked responses (AER) with administration of amphetamine in children and in patients with affective disorders. Individual differences are prominent both in behavioral response as well as the AER; some individuals are aroused and others show sedation or no effect and AERs both predict and parallel these differences. These studies have relied on oral administration of the drug but it has not been possible to follow blood levels.

Do these individual differences result partially from differences in absorption or pharmacokinetics? With the availability of blood amphetamine level measures this question may be studied. A series of 10 children who met rigorous diagnostic criteria for hyperactivity have been studied with serial AERs, blood amphetamine levels and behavioral ratings at two hour intervals post single dose amphetamine administration. AER data following chronic amphetamine have also been collected. Preliminary analysis of the data revealed a correlation of -0.72 betwen blood amphetamine level at 2 hours and a newly developed measure of AER variability. This association of low variability and high blood amphetamine level is consistent with the amphetamine stereotypy concept. Further data has been collected on nine children following both placebo and amphetamine administration.

During the next year, additional children will be studied and the mathematical tools further developed with various computer simulation techniques.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00111-03 BP

PERIOD COVERED
July 1, 1975 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Drug Abuse Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

William F. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BPB NIMH
Glenn C. Davis, M.D.	Clinical Associate, Unit on Drug Abuse	BPB NIMH

OTHER:

Robert M. Post, M.D.	Unit Chief, Section on Psychobiology	BPB NIMH
Daniel B. van Kammen, M.D.	Unit Chief, Section on Neuropsychopharmacology	BPB NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Science, NIMH
Laboratory of Clinical Pharmacology, NIMH
Laboratory of Clinical Neuropharmacology, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed towards a basic understanding of the mood alterations induced by drugs of abuse. Three areas are under investigation: the "high" produced by intravenous amphetamine and cocaine; the mood effects of endogenous opiate-like substances; the affective component of the experience of pain and its alteration with various pharmacological agents.

Other Investigators Continued:

Gerald LaVonne Brown, M.D.	Medical Officer	BPB	NIMH
Dennis L. Murphy, M.D.	Chief, Clinical Neuropharmacology	CNB	NIMH
Monte Buchsbaum, M.D.	Unit Chief, Perceptual and Cognitive Studies	BPB	NIMH
Richard J. Wyatt, M.D.	Chief, Laboratory on Clinical Pharmacology	SMR	NIMH

Objectives:

(1) Drugs of abuse such as cocaine and amphetamine produce profound alterations in mood. There are major differences in acute and chronic administration. Our studies are designed to obtain a neurochemical understanding of these alterations.

(2) Drugs of abuse bring about long term behavioral effects when used chronically. Are these changes related to neurotransmitter-receptor changes?

(3) Narcotic analgesics are major drugs of abuse. Recent discovery of endogenous opiate-like substances (endorphins) have stimulated the search for the physiological role and possible participation of these substances in pathological states such as schizophrenia. We are determining the role of endorphins in normal physiology as well as investigating their possible participation in schizophrenia and affective illness.

(4) Pain has a major affective component. We are studying how affectively ill patients experience pain both in their subjective reports and in experimental studies. Through use of the narcotic antagonist naloxone, we are investigating the normal participation of endorphin in pain regulation.

(5) This project continues to develop a treatment program for drug abuse.

Methods Employed:

(1) Patients who meet criteria for drug abuse, stimulant type are admitted to the Clinical Center wards 2-West and 3-West for study and intensive treatment.

(1.1) Treatment Includes:
Drug withdrawal and detoxification if necessary.
Careful medical evaluation for complications of chronic drug use (e.g., hepatitis).
Intensive individual psychotherapy, psychotherapy is goal oriented towards individual needs and a cognitive understanding of the function that drug use serves in the individual.
Careful nursing intervention especially help dealing with common life-style problems that the drug abuser manifests on the ward.

(1.2) Historical and Daily Behavioral Data:
Extensive histories of drug use are obtained

including type, frequency, chronicity, mixing and subjective effects. Behavioral data are obtained in a ward situation on mood and symptoms as indicated.

(1.3) Infusion Studies:
Much of the drug abuse studies center around the administration of drugs. Amphetamine and or cocaine are administered in nontolerant patients (in doses that are less than the patients were previously habituated to). Behavior is rated by trained investigators along various dimensions. Blood and urine are collected for amphetamine or cocaine levels. Measurements of various catecholamine and their metabolites are carred out as well. Studies are done in collaboration with the Laboratory of Clinical Science. Lithium and alpha-methyl-para-tyrosine (AMPT) are administered in a placebo controlled double blind fashion to study the alterations subjective effects and biochemistry.

(2) Patients admitted on 2-West, 3-West, 4-East and at St. Elizabeth's (in treatment programs for affective illness and schizophrenia) are administered naloxone intravenously in double blind placebo controlled fashion to determine whether endorphins play a role in these illnesses. Studies with the narcotic antagonist, naltrexone are planned.

(3) In collaboration with the Unit in Perceptual and Cognitive Studies, we carry out studies on pain utilizing mild electric shocks (1-31 ma, .001 second duration), subjective ratings and average evoked potentials. Affectively ill subjects hospitalized on 3-West participate in these studies. Normal subjects have been involved utilizing the same shock/evoked potential proceedure but studied with aspirin and naloxone. Other analgesic studies are planned.

Major Findings:

(1) The amphetamine and cocaine projects are not complete at this time. 8-10 patients have been admitted for study.

(2) Fourteen schizophrenic patients and five patients with affective disorders were given naloxone (0.4-10mg.) or placebo intravenously in a double-blind fashion. Physicians' ratings of hallucinations, mannerisms and posturing, conceptual disorganization, psychosis and mood did not change significantly. A single item, unusual thought content, improved significantly on the naloxone day compared to the placebo day. There was no improvement in mood in affectively ill patients

either as self-rated or rated by physicians. Naloxone did not produce marked improvement in any patient studied, suggesting that the acute blockade of opiate receptors is not associated with global improvement in psychotic symptomatology.

(3) Preliminary data suggests that affectively ill patients experience less pain than normals. There are major bipolar unipolar differences. Also preliminary results suggest that naloxone does affect pain appreciation though the direction is dependent upon pain sensitivity.

Significance to Biomedical Research:

(1) Drug abuse is a significant problem. A basic understanding of the mechanisms of action of drugs of abuse could lead to pharmacologic agents useful in prevention, detoxification, withdrawal and long term treatment.

(2) Endorphins may play an important physiological role in man. Pathological states may be related to endorphin disorders. Investigations thus have both basic and treatment orientation.

(3) Affective illness and schizophrenia have major alteration in the appreciation of painful stimuli. Research in this area may uncover basic mechanisms in pain regulation as well as suggest treatment strategies.

Proposed Course:

All studies are ongoing.

Publications:

Davis, G.C., Bunney, W.E., Jr., DeFraites, E.G., Kleinman, J.E., van Kammen, D.L., Post, R.M., Wyatt, R.J. Intravenous naloxone administration in schizophrenia and affective illness, Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00070-04 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Psychological and Biological Interactions in the Acute Psychoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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12.0	6.0	6.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is involved in the intensive, longitudinal study of both psychological and biological phenomena in a spectrum of acute psychiatric illnesses, including depression, mania, schizoaffective illness, and drug-induced psychoses. The major research strategies include: precise longitudinal behavioral and biological measurements in the context of double-blind clinical trials of both routine and new, specific pharmacological agents. Amines, metabolites enzymes, electrolytes, cyclic nucleotides, and related biological regulatory compounds are measured in body fluids of patients on and off medications and in different phases of illness and recovery. Motor activity, sleep, and averaged evoked responses are also monitored as well as specific measures of mood, cognition, abstraction, and verbal learning. An active animal and primate laboratory is maintained for the development of new techniques, conducting behavioral-biological studies not feasible in man, and studying animal "models" of psychosis, such as chronic cocaine-induced behavioral changes in the monkey and electrical kindling. An active interplay of psychological and biological perspectives thus occurs in seeking and developing new theories, research and treatment approaches for a range of psychotic illnesses in man.

I. Project Description:

A. Objectives: The project is engaged in the multidisciplinary, longitudinal study and treatment of patients with a spectrum of acute psychoses, particularly involving mood disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interactions.

B. Methods Employed:

1. Patients who meet formal research diagnostic criteria for manic-depressive illness, schizoaffective illness, schizophrenia, and drug-induced psychoses are admitted to the Section on Psychobiology, 3-West Clinical Research Unit. Normal volunteers are also admitted to provide control data for various psychological and biological projects.

2. During an initial drug-free interval, patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. Treatment and clinical evaluation are begun in individual and group psychotherapy as well as ongoing clinical case conferences. Behavioral data are collected twice daily, both by a trained nursing team and by patient self-ratings. Nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly. Motor activity is assessed in collaboration with Dr. Theodore Colburn utilizing a miniaturized activity monitor wo on the nondominant wrist.

3. Clinical and neuroendocrine parameters are evaluated during testing with apomorphine, L-DOPA, amphetamine, and other agents such as thyrotropin releasing hormone.

4. Amine metabolites and electrolytes are systematically evaluated utilizing urinary, blood, and cerebrospinal fluid approaches to neurotransmitter metabolism.

5. Both routine and experimental compounds are evaluated during double-blind clinical trials.

6. The transient antidepressant effects of one night's sleep deprivation in patients with severe depressive mood disorder is examined and underlying neurobiological and neuroendocrine changes examined.

7. Animal Models. A rodent and primate behavioral pharmacology laboratory is maintained in order to develop new research techniques in the longitudinal study of biobehavioral interactions. Particular focus is given to the study of chronic cocaine-induced behavioral pathology and the underlying changes produced by repetitive stimulation of the amygdala (electrical kindling).

C. Major Findings:

1. Cerebrospinal Fluid Studies as an Approach to Central

Neurotransmitter Metabolism (in collaboration with Frederick Goodwin, Edna Gordon, and David Jimerson).

a. CSF Norepinephrine. In collaboration with Dr. Ray Lake, CSF norepinephrine has been measured utilizing a sensitive radioenzymatic assay in patients with manic and depressive illness. It was found that manic patients have significantly higher baseline and probenecid-induced accumulations of norepinephrine than do depressed patients or neurological controls. Increased psychomotor activity accompanying mania may be a partial explanation for these findings. However, some manic patients who were maintained on strict bedrest had markedly elevated CSF norepinephrine and the relationship of these elevated levels to the manic syndrome remains a promising lead for further exploration. Values of norepinephrine in depression were not significantly different from controls. Patients with high anxiety did have significantly higher levels of norepinephrine compared to depressed patients with low anxiety, however. Since CSF norepinephrine does not appear to be derived from peripheral metabolism these data represent one of the first direct tests of the catecholamine hypothesis, possibly implicating elevated levels of norepinephrine in mania.

b. Cerebrospinal Fluid Dopamine Metabolites. While depressed patients do not appear to have significantly different levels of the dopamine metabolite homovanillic acid (HVA) in cerebrospinal fluid compared to other psychiatric comparison populations, the range of HVA values may be clinically relevant. Preliminary studies suggest that patients with evidence of low HVA accumulations following probenecid administration (a measure of dopamine turnover) show the best response to treatment with a dopamine receptor agonist such as piribedil. Similarly, patients with low baseline HVA levels responded with the greatest antidepressant effects to the sleep deprivation paradigm. Thus, patients with evidence of low central dopamine function may be differentially responsive to clinical antidepressant strategies even though the overall HVA in depressed patients is not significantly different from controls.

c. CSF Serotonin Metabolism and Subgroups of Patients With Affective Illness. In collaboration with Frederick Goodwin, preliminary data suggest that patients with low serotonin turnover, as estimated by accumulation of 5-hydroxyindoleacetic acid (5HIAA) following probenecid, may help predict subsequent pharmacological responsivity. That is, unipolar depressed patients with high 5HIAA accumulations tend to be better antidepressant responders to the drug imipramine. These patients with high 5HIAA in CSF have significantly lower norepinephrine metabolism as estimated by levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) in urine.

d. Thus, the preliminary data available for serotonin, norepinephrine, and dopamine metabolites suggest that depressed patients with different biological abnormalities may be differentially responsive to pharmacotherapy and that establishing a given patient's biological profile, which may be independent of clinical phenomenology, may ultimately have important clinical and therapeutic implications.

2. Cerebrospinal Fluid Electrolytes:

a. CSF Calcium. Work initiated in collaboration with John Carman has been continued with David Jimerson, and several consistent findings have emerged. While levels of CSF calcium in depressed patients are not significantly different from neurological controls, there is a significant positive correlation of severity of depression in CSF calcium measured in either baseline 9:00 a.m. samples or at 3:00 p.m. following probenecid administration. These correlational data suggesting that more severely depressed patients have higher CSF calcium is consistent with paired data in individual patients demonstrating rapid mood cycles. In these patients CSF calcium levels are significantly higher during the depressed phases of illness than during the manic phases.

b. A variety of treatment strategies also appeared to decrease CSF calcium in association with antidepressant response. As previously reported, successful treatment of severely depressed patients with electroconvulsive therapy is associated with a significant decrease in CSF calcium. The degree of improvement in depression during lithium therapy was correlated with the degree of decrease in CSF calcium. There also was an interaction of sleep deprivation response with CSF calcium change--those improving had decreases in CSF calcium while those who became worse had increases in calcium. Seven of eight depressed patients restudied during a medication-free interval, likewise, showed decreases in CSF calcium compared to levels when they were previously depressed. In schizophrenic patients studied in collaboration with Daniel van Kammen, recovery from an acute schizophrenic episode was associated with a significant increase in CSF calcium. CSF calcium in medicated patients with seizure disorders studied in collaboration with James Wood tended to be lower than neurological controls. These data on CSF calcium in a variety of clinical states are consistent with the experimental literature suggesting that decreases in CSF calcium are associated with increased neuronal irritability while increases in CSF calcium tend to act as an anticonvulsant and decrease behavioral arousal. Several different clinical approaches suggest an association between CSF calcium and mood disturbances--higher levels of calcium with more severe depression and decreased levels following clinical improvement with a variety of strategies.

c. Dopamine B-Hydroxylase (DBH) (in collaboration with Pauline Lerner, Walter Lovenberg, and Frederick Goodwin). DBH has been measured in cerebrospinal fluid of manic and depressed patients. In contrast to the higher levels of norepinephrine observed during mania, DBH levels were significantly lower in this clinical state.

d. Cerebrospinal fluid levels and accumulations following probenecid of cyclic AMP and cyclic GMP studied in collaboration with Craig Smith and John Tallman were demonstrated to be not significantly different across a variety of psychiatric diagnoses. Concentrations of these "second messengers" also were not significantly altered by several psychotropic medications, including lithium. Cerebrospinal fluid gaba-amino-butyric acid (GABA) levels studied in collaboration with Candace Pert and Robert Horlick

were also found not to differ across diagnostic psychiatric categories.

e. Polypeptides in CSF. In collaboration with Candace Pert, substances with opiate-like activity are being measured in the spinal fluid of patients with manic and depressive illness. Naloxone has been reported to reverse the marked behavioral effects of intraventricularly administered endorphins in animals. A preliminary trial of naloxone in patients with affective illness (with Glenn Davis) showed that naloxone was not associated with significant therapeutic effects. With David Jimerson and Jay Skyler, prolactin has been measured in cerebrospinal fluid and has been shown to decrease following treatment with a dopamine receptor agonist, piribedil, and to increase following neuroleptic administration with pimozide.

f. Several laboratories have studied red blood cell/plasma ratios of lithium as a model for lithium transport in the central nervous system. In an attempt to examine another aspect of central nervous system lithium mechanics, we have measured lithium levels directly in CSF and compared them to those in plasma. Patients studied acutely after approximately one week of lithium treatment have significantly lower CSF lithium levels than those studied after four weeks of lithium administration. CSF plasma lithium ratios are more variable during the acute phase of administration than during chronic treatment and the ratio tends to stabilize at approximately 0.38 mEq/l. The absolute levels of lithium in blood or CSF at either the acute or chronic time interval were not predictive of clinical efficacy, however. The time lag before stable CSF lithium levels are achieved during lithium therapy may help explain the lag in onset of lithium's clinical effectiveness.

3. Dopaminergic Mechanisms in Depression and Mania:

a. Agonist and Antagonist Strategies. A variety of animal laboratory and clinical data in man suggest that dopaminergic mechanisms are importantly involved in some schizophrenic-like psychoses. Less well appreciated is the possible role of dopamine in affective illness. While the results reviewed below from our laboratory are suggestive of a role for dopamine in the mood disorders, we would not suggest an exclusive or specific involvement of dopamine; other neurotransmitter systems discussed above may also be importantly involved.

b. Piribedil, a Direct Dopamine Agonist. Treatment of severely depressed patients with piribedil in doses up to 240 mg/day has been associated with a range of therapeutic responses. Some patients showed mild clinical improvement while others showed substantial clinical change; one depressed patient became worse. This range of antidepressant responsivity appeared to be predicted by pretreatment accumulations of the dopamine metabolite HVA in cerebrospinal fluid. The strength and specificity of this finding remains for further exploration.

c. Amphetamine, an Indirect Catecholamine Agonist. With David Jimerson and Victor Reus, a clinical trial of amphetamine has been conducted in depressed patients. Following treatment with intravenous amphetamine (20 mg.)

during a double-blind diagnostic clinical interview, a range of mood responses was again demonstrated. Some patients showed improvement while others became more dysphoric. The degree of clinically rated dysphoria was negatively correlated with 5HIAA accumulations in cerebrospinal fluid. These data are consistent with several studies in animals suggesting that decreasing serotonergic function may be associated with potentiation of responses to psychomotor stimulants. Conversely, increasing serotonergic function may decrease responsiveness to catecholaminergic stimulation. These data are consistent with our current clinical findings suggesting that those patients with the most physiological activation experienced the most dysphoria following amphetamine. An electrophysiological measure, the averaged evoked response, studied in collaboration with Monte Buchsbaum was also associated with amphetamine response. Those patients who were reducers (decreased amplitude of evoked potential with increasing intensity of stimulation) responded with mood elevation while those patients with an augmenting pattern (increased amplitude of evoked potential with increasing intensity of stimulation) tended to be more dysphoric. Mood response during an amphetamine interview was likewise correlated with neuroendocrine responsivity. Those patients who had the largest growth hormone response to L-DOPA administration experienced dysphoria following amphetamine administration. These data suggest that amphetamine may help uncover underlying biochemical, neuroendocrine, and electrophysiological differences in responsivity which may be important in defining subgroups of depressed patients.

c. A Relatively Specific Dopamine Receptor Antagonist Pimozide in Mania. A continuing trial of pimozide and the related neuroleptics in manic illness also supports a role for dopamine in this syndrome.

d. Paradoxical Effects of Low Dose Treatment with a Dopamine Receptor Agonist Piribedil. Several laboratory studies have suggested that in low doses the dopamine agonist may have inhibitory effects on behavior rather than the typical findings of hyperactivity and stereotypy following high dose administration. Two of three manic patients appeared to have substantial clinical improvement during treatment with low dose piribedil (60 mg/day).

e. These data and a variety of other studies in the literature suggest that treatment with agents that affect dopaminergic systems may have important effects on mood disturbances. Direct- and indirect-acting dopamine agonists may be associated with a range of antidepressant responses while inhibition of dopaminergic systems (either directly through postsynaptic receptor blockade or indirectly with presumed preferential presynpatic receptor stimulation) may possibly be associated with antimanic and antipsychotic effects. These data, taken with those implicating dopaminergic mechanisms in the schizophrenic syndromes, suggest that dopaminergic mechanisms may be involved in a variety of neuropsychiatric disturbances and may not be specific to a given illness. Considering the time course of alterations and the possibility of a regional specificity may help clarify the relationship of dopamine to various psychiatric syndromes.

4. Studies of the Sleep Deprivation Effect in Depressed Patients:

a. A Model for Acute Mood Alteration in Depression. Transient antidepressant responses continue to be observed in the most severely depressed patients following one night of total sleep deprivation. Study of these phenomena may yield important leads to underlying biophysiological changes associated with acute alterations in mood. For example, responders and nonresponders to the sleep deprivation paradigm have differential circadian variation in temperature. Those depressed patients who improved following sleep deprivation continue to show the usual diurnal variation with afternoon increases in temperature, while nonresponders have flat temperature curves the day following sleep deprivation. As expected, normal volunteer controls (studied by Robert Gerner) respond to one night's sleep deprivation with mild increases in fatigue, irritability, and dysphoria in contrast to the antidepressant effects in depressed patients. The volunteers who show the most dysphoria tend to have flat temperature curves both prior to and during the sleep deprivation procedure, again suggestive of altered circadian rhythmicity in relation to this mood response.

b. Following sleep deprivation, depressed patients showed a pattern of greater augmentation on averaged evoked potentials (with Monte Buchsbaum). They also tended to have increases in morning plasma cortisols in contrast to nonresponders and normal volunteers. The degree of antidepressant response to sleep deprivation was correlated with the baseline levels of HVA in CSF but not with the probenecid-induced accumulation of HVA. Those who showed the most improvement following sleep deprivation had the lowest pretreatment HVA levels.

c. David Jimerson, in collaboration with Drs. Lynch and Wurtman at MIT, has examined the role of circadian rhythms of melatonin excretion in relation to antidepressant response. Both normal volunteers and depressed patients have similar and marked diurnal changes in urinary melatonin, excreting large amounts during the evening hours and reduced amounts during the awake daytime periods. One night's sleep deprivation does not change this diurnal variation in melatonin excretion in either the depressed patient or normal volunteer group. However, preliminary studies in normal volunteers suggest that the diurnal variation in melatonin excretion may be altered by longer phase reversals of sleep-wake cycles.

d. Studies with Herbert Weingartner have documented that the mood alterations accompanying sleep deprivation are associated with state-dependent effects on learning and memory.

5. Mechanisms in Rapidly Cycling Manic-Depressive Patients:

a. Reliable clinical, biochemical, and electrophysiological changes have been documented in patients with rapidly cycling manic-depressive illness. For example, intensive longitudinal case study has revealed consistent significant differences in urinary MHPG excretion in the depressed compared with the manic phase. These alterations in the norepinephrine metabolite (thought to partially reflect central sources of norepinephrine metabolism)

were associated with the mood change and did not anticipate it. The alterations in urinary MHPG did not directly vary with degree of psychomotor activation or retardation measured by telemetric activity monitoring devices, and may thus be an important concomitant of the mood shift. One depressed patient, studied in collaboration with Christian Gillin, consistently switched into a manic phase during the rapid eye movement phase of sleep. This is the first report of EEG documented switches occurring during the REM episode. Dr. Buchsbaum has shown that some evoked potential measures change concomitantly with the mood change prior to the switch and may be associated with important underlying mechanisms associated with the triggering process.

b. In collaboration with John Carman, alterations in serum electrolytes have been studied through the switch process in patients with rapid cycles into mania or psychosis. He finds statistically significant increases in serum total calcium acutely at the time of the switch process.

6. Laboratory Studies of Chronic Sensitization Phenomena:

a. Animal Models for Affective Illness and Schizophrenia (in collaboration with Kathleen Squillace and Agu Pert). Repetitive administration of the same dose of cocaine is associated with increased effects on hyperactivity and stereotypy, and may also be associated with an increased susceptibility to cocaine-induced seizures. This increased susceptibility to convulsive activity led us to study the interaction of pharmacological sensitization phenomena with the electrical kindling paradigm. Kindling involves daily electrical stimulation of various limbic sites, particularly the amygdala, with current which is initially subthreshold for EEG or behavioral effects. Repetition of this stimulation (200 microamps, 50 Hz/500 milliseconds) eventually is associated with increasingly long amygdala after-discharges and finally culminates in the animal having a major convulsive seizure.

b. Recent studies have documented that amygdala kindling in this fashion alters behavioral responsivity to several drug challenges. Kindled animals compared to implanted sham-stimulated controls have significantly decreased cocaine-induced vertical hyperactivity; they also have a significantly higher incidence of lidocaine-induced convulsions than do nonkindled controls. These data suggest that relatively long-term alterations in behavioral responsivity and in convulsive threshold may be achieved with electrical kindling. This procedure may represent a useful strategy for producing long-term alterations in excitability of specific substrates important in the regulation of emotional and cognitive behavior.

c. In three separate amygdala kindling studies, approximately 40% of the animals developed marked oscillations in after-discharge duration. Some animals showed a tendency to 48-hour cycles, i.e., they demonstrated long after-discharges lasting approximately two minutes on one day and short after-discharges of ten to thirty seconds on the next, and so on. These differences in after-discharge duration were also associated with the appearance of major seizures on the days when long after-discharges were present. It is suggested that these marked oscillations in after-discharge duration

reflect basic rhythmic alterations in amygdala excitability which may be of behavioral consequence and may be a useful model for other central nervous system cyclic phenomena. Preliminary studies suggest that lithium did not alter the incidence of development of the oscillatory pattern, however.

d. The rhesus primate also tends to show increasing responsivity to the same dose of cocaine following repeated administration. Possible mechanisms underlying this phenomenon are being explored using assessment of cerebrospinal fluid amines, metabolites, cyclic nucleotides, and electrolytes. Chronic studies of psychomotor stimulant administration in animals may help focus on important time-dependent behavioral and biological changes of relevance to the development of psychopathology in patients even if the models are incomplete and the behavioral pathology induced is not analogous in the human.

7. Studies of Cognitive Function in Depression (in collaboration with Drs. Earl Silber, Alix Rey, and Robert Savard).

a. A rating scale has been constructed to measure deficits in both the form and content of language in depressed patients. Paradoxically, the most severely depressed patients appear to have an inability to express depressive affect and have increased deficits in formal aspects of language.

b. Dr. Rey and Dr. Savard have studied the Halstead Categories Test in 22 depressed patients, 24 normal controls, and 12 spouses of the depressed patients who represent a closely age and socio-economically matched comparison group. Significant deficits in this measure of abstracting ability were found in the depressed patients compared to either control group. Patients made more errors on first testing during the depressed state, as well as on repeat testing during clinical improvement.

c. Clear identification of various cognitive and language deficits in depressed patients may help in the eventual definition of depressive subtypes which may in turn relate to different biological mechanisms involved. Similarly, there may be an active interplay between concepts explored in the laboratory and those in the clinic. In collaboration with Arnold Meyersburg, we have examined the parallelism between various psychological and neurobiological approaches to the developing organism. Several psychoanalytical concepts including fixation, regression, and repression may be usefully reexamined in light of newer neurobiological concepts. The electrophysiological model of kindling may also be helpful in exploring psychological sensitization mechanisms of importance in the development of psychopathology.

D. Proposed Course of the Project:

1. Several drugs which are active in our laboratory models will be studied in patients with manic-depressive illness and schizophrenia. For example, the anticonvulsant carbamazepine (Tegretol) inhibits kindling and is currently the drug of choice in patients with psychomotor seizures. It also has been reported to have salutary effects in patients with associated mood

disorders. Of three manic patients tested to date (with James Ballenger), one who was previously shown to be a lithium nonresponder, showed a clear response to carbamazepine. He worsened following placebo substitution and again improved when carbamazepine was restarted. Another anticonvulsant, diazepam, may have some spectrum of antipsychotic effects when administered in high doses (with David Jimerson and Daniel van Kammen). Exploration of these and related compounds may provide alternate strategies to the accepted major treatments of manic-depressive illness and schizophrenia, such as lithium and neuroleptics, particularly for nonresponders to these routine strategies. Clinical trials of drugs with relatively specific effects at catecholamine receptor sites will also continue.

2. In addition to continued exploration of the cerebrospinal fluid strategies as an approach to central neurotransmitter mechanisms, the neuroendocrine approach will also be studied in detail. Studies of neuroendocrine responsivity in response to challenges with apomorphine, L-DOPA, and releasing factors such as TRH will be continued in collaboration with Dr. Jimerson, Dr. Rey, Dr. Ballenger, and Dr. Gold. Detailed exploration of the pituitary adrenal axis will also be conducted in collaboration with Victor Reus and Pauline Lerner.

3. Thus, the work on the unit will continue to attempt to integrate the multiple psychological and biological perspectives in the study of treatment of patients with a spectrum of severe psychiatric disorders and especially the mood disorders. Basic to this approach is the study of genetic and experiential predispositions and biological indicators as they may be separated from state-dependent biological changes and those secondarily related to the illness. As in the animal models discussed, particular attention will be paid to longitudinal changes in both the clinical phenomenology and in underlying biochemical changes since much evidence suggests that alterations during an acute episode are very different than those involved later in the illness. The differential effects of acute and chronic drug administration will also be explored in relation to clinical efficacy. Time-dependent effects on an holistic behavioral level as well as on a molecular level stressing adaptive regulatory mechanisms will thus be explored in relationship to the development of psychiatric pathology and its treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00049-03 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Psychosocial Factors in the Prognosis of Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the effect of various psychosocial factors on host resistance to malignant melanoma. A detailed characterization of these factors in patients with melanoma, Clinical Stage II, is being obtained. In addition certain endocrine and immune parameters are also being studied. We are testing several hypotheses which may distinguish those patients suffering recurrence of their disease from others who either remain disease-free or have a delayed recurrence. At one year, significant correlations with disease-free status were found with 1) number of positive lymph nodes, 2) ABO type, 3) social assets, and 4) melanoma adjustment self rating score.

PHS-6040

Project Description

Objectives

This study is aimed at developing a detailed characterization of the psychosocial factors which distinguish those patients who suffer recurrence of malignancy from those who remain disease-free or who have a delayed recurrence. Data is being gathered to test several current hypotheses concerning psychosocial antecedents of the onset of physical illness and predisposing psychobiological states.

Fifty to 100 patients with "surgically-cured" Stage II Melanoma will be studied. We plan a multi-level assessment of each patient. In addition to the biological measures gathered on the patient, we will characterize the patient's genetic history, past psychosocial history, level of personality development, current life stress and present psychological state. We will accomplish this by (1) psychiatric interview; (2) objective assessment of a 5-minute speech sample, and (3) standardized questionnaires including MMPI, Holmes and Rahe Schedule of Recent Experience, SCL-90, Rotter Internal-External Locus of Control Scale, and Beck Depression Inventory.

The patients are seen shortly after their "curative" surgery and will be followed up at 2-month intervals, at which time we will obtain the Holmes-Rahe Scale, the SCL-90, Helplessness-Hopelessness Index and Beck Depression Inventory.

At one year, significant correlations with disease-free status were found with 1) number of positive lymph nodes, 2) ABO type, 3) social assets, and 4) melanoma adjustment self rating score. We are presently examining this finding in a second group of patients. A paper was presented at the American Psychosomatic Society.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00132-03 BP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biological and psychopharmacological evaluation of schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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12	10	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental and clinical findings have indicated that schizophrenia is a heterogeneous psychiatric disorder with biochemical, psychophysiological, psychological and genetic components. Pharmacological treatment is a particularly effective intervention, but not a cure for all patients. Because of its very nature, this disorder is difficult to study. Patients are not constantly psychotic and their clinical state may change, regardless of medication conditions. However, we are now able to study a schizophrenic patient population intensively and longitudinally, with and without pharmacological approaches during different clinical states. We are beginning to ask fundamental questions about the nature of schizophrenia, indications for pharmacological treatment, vulnerability to psychotic decompensation and the role that genetics may play in the transmission of the disorder.

In the United States alone, it is estimated that 2 million people suffer from schizophrenia. Our work integrates the multidisciplinary approaches in an effort to understand this complex disorder which may lead to a more effective treatment.

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The Section on Neuropsychopharmacology (1) plans and conducts multidisciplinary research on the etiology and treatment of schizophrenia and (2) conducts research to define the biological dysfunction in schizophrenia.

Objectives

1. Multidisciplinary research on the etiology, treatment and prevention of schizophrenia
2. Evaluation of the dopamine hypothesis of schizophrenia
3. Evaluation of potential markers of vulnerability and predictors of antipsychotic drug response
4. Psychopharmacological and biochemical evaluation of trait- and state-dependent variables of schizophrenia

Methodology

One to 14 days after admission, patients are taken off their neuroleptic medication and put on placebo. After this 2-week "washout period," patients go through a 3- to 4-week test period in which diagnostic evaluation, medical and neurological workup, cerebrospinal fluid (CSF) collections, drug infusions (amphetamine, naloxone, placebo), psychological and psychophysiological testing and neuroendocrine evaluations take place. These are followed by two or more drug trials which include pimozide, lithium, diazepam, GHB or lioresal. During any of these drug trials, testing similar to the admission test week can take place. Each drug period is separated by a 2-week placebo "buffer" period. The last of this period is either followed by another test period or immediately followed by a 4-week clinical period where, if indicated, the patient is put on generally available antipsychotic medication and prepared for discharge. This extensive data-collection system allows us to evaluate the individual variables and drug responses against one another and to observe the patient in different clinical states. It will enable us to develop psychosocial, endocrine, biochemical, and pharmacological profiles on patient groups defined by each variable.

A major problem of schizophrenia research has always been certainty of diagnosis. In addition to using the International Pilot Study of Schizophrenia criteria as developed by the World Health Organization and the former Psychiatric Assessment Section of the Adult Psychiatry Branch, we are applying the Research Diagnostic Criteria as described by Dr. Spitzer and his collaborators to define diagnostically our patient population.

The 4-East Nursing Staff has been trained in the application of the Bunney-Hamburg psychosis, depression, and mania scale, and we have added a scale to measure extrapyramidal side-effects (EPS) and sedation.

Further, the scale of psychotic disorganization as designed by Drs. Docherty and van Kammen has been used by the 4-East Nursing Staff, and initial reliability and validity have been established. Understanding of the stages of phenomena may strengthen our interpretation of biological data. Dr. Docherty presented a paper about the stages of psychotic disorganization at the 1977 World Psych-

In collaboration with the Section on Biochemistry, BPB, new assays for measuring peptides in spinal fluid have been started. This coming year, for the first time, we have drugs available that are relatively specific for the different neurotransmitter systems that we are studying.

Major Findings: Pharmacological

A. Dopamine Stimulation

d-Amphetamine, an indirect dopamine receptor agonist, has been shown to worsen some acute psychotic symptoms. At the same time, numerous clinical reports of increased social contact and therapeutic effects have appeared in the psychiatric literature over the last 25 years. This, therefore, seems a potentially crucial area of investigation for clarifying the limits and nuances of the dopamine hypothesis. Patients receive I.V. amphetamine and I.V. placebo during the initial test period. This is repeated during pimozide withdrawal and/or lithium.

A standardized rating system has been developed for quantitating behavioral change before, during and after the infusion interview. Additionally, blood samples are taken regularly during the day to monitor amphetamine blood levels, serum prolactin, norepinephrine, and DBH levels. Clinically, schizophrenic patients appear to respond differently to amphetamine than depressed patients, although there is heterogeneity within each group (a paper has been published). Strikingly, the depressed patients do not become more psychotic, nor do patien with a schizophrenic diagnosis as a group. However, within this schizophrenic group the more severely psychotic patients may improve, while some patients in early remission may get worse. Serum norepinephrine is raised 15 minutes after the acute amphetamine administration. This rise over baseline is not affected by either pimozide or lithium. Serum DBH does not seem to be affected by amphetamine. This suggests that amphetamine affects newly synthesized, rather than stored, norepinephrine. However, there may be a competitive inhibition of DBH by amphetamine. Comparative animal data are not available. Lithium, which is supposed to prevent stimulation-induced release of norepinephrine, had no effect upon the amphetamine-induced rise in norepinephrine. Dr. Rosenblatt presented this work in collaboration with Drs. Lake, Ziegler and Bunney at the 1977 American Psychiatric Association Annual Meeting. A paper has been submitted for publication. Prolactin levels were increased 25 and 40 min. postamphetamine infusion. This was rather striking since the placebo infusions which took place at the same time of the day--between 8-9 a.m.-- showed a slight decrease from the rise of prolactin during sleep as a result of diurnal variation. This is in contrast to the expected decrease in prolactin levels following increased dopamine activity. The increase is hypothesized to be due to stress or activation of serotonin mechanisms in the hypothalamus and pituitary. This needs to be followed up with lower dose amphetamine prolactin studies, as well as in other diagnosed populations. A paper was presented at the 1977 American Psychiatric Association Meeting. We observed that depressed patients did not show an increase in pulse within the first 10 min., while schizophrenic patients did rather consistently. Both groups showed a similar rise in blood pressure. A paper has been submitted

for publication. Z01 MH 00132-03 BP

B. Dopamine Receptor Blockade and Schizophrenia

(a) Pimozide in acutely psychotic patients - Following a washout period, patients are started in a double-blind, placebo-controlled trial of the effects of pimozide in acutely psychotic and remitted patients. This antipsychotic agent is of especial interest because of its relatively specific postsynaptic dopamine receptor blocking effect.

During this project endocrine studies, amphetamine infusion, sleep and CSF studies are conducted. Selected patients are put on sleep study to evaluate the effects on sleep. In collaboration with Dr. Gillin we have shown that pimozide prevented amphetamine having any effect on sleep. Rapid eye movement sleep decrease is completely prevented by pretreatment of pimozide. This is in contrast to the behavioral effects of amphetamine, which are reversed in the people who improve while drug-free after amphetamine and who then, after pimozide, become more psychotic. We also gave amphetamine infusions seven days after pimozide discontinuation to evaluate whether receptor hypersensitivity could be connected with a change in behavioral response. Data from this project are presently under analysis. However, we did not observe a strong response to amphetamine, which questions the dopamine hypersensitivity hypothesis of psychosis. In collaboration with Drs. Gillin, Post and Bunney, a paper evaluating the effects of pimozide on sleep in psychiatric patients has been submitted for publication, as well as a paper on the pimozide attenuation of the amphetamine-induced REM suppression.

Other studies include endocrine studies, cyclic AMP in CSF, effects of pimozide on DBH in spinal fluid, metabolites of dopamine, norepinephrine and serotonin in the spinal fluid and 24-hour urine collections which take place in addition to the standard battery of behavioral and state of illness ratings.

In a first group of 13 patients the increase in prolactin observed after pimozide and the decrease in psychosis were correlated. However, in a second group of five male patients, this was not observed. In collaboration with Dr. Heykants of Beerse, Belgium, we are measuring pimozide levels in our patients with a radioimmunoassay.

C. Use of Antidepressants in Schizophrenia

(a) An extensive review of the literature was conducted to ascertain the experience which has been accumulated in using tricyclic antidepressants and monoamine oxidase inhibitors in the varied types of schizophrenia. Although the number of well-controlled studies in this area is limited, it is well-documented that antidepressant medications are widely prescribed for these patients. A paper has been submitted for publication.

We observed that eight of a group of 24 psychotic patients, whom we were not able to differentiate between on admission, remitted when their antipsychotic medication was withdrawn over a 30-day period. Although these patients all had been diagnosed schizoaffective schizophrenia according to

RDC classification (1975), this diagnosis did not differentiate the drug-free remitters from the schizoaffective nonremitters. The drug-free remitters appeared to have a later age of onset, a shorter time of hospitalization and better premorbid functioning. The drug-free remitting male patients had low platelet MAO activity below the mean for normals, while the nonremitting patients had platelet MAO activity ranging from high to low. The drug-free remitters also responded to lower than the suggested dose ranges for the nondrug-free remitters. This continued to be present at one-year follow-up (150 vs. 484 chlorpromazine units/day). This finding may lead to a differential approach of medicating acutely psychotic patients. It suggests that if acutely schizophrenic patients have not responded with improvement to neuroleptic drugs at 4-6 weeks, the dose may need to be decreased rather than raised. This finding needs to be examined further.

(b) Lithium in schizophrenia

Since the behavior activation induced by amphetamine also was attenuated by it, lithium may have some antidopamine effects. We studied lithium carbonate in psychotic and remitted schizophrenic patients: Nine of thirteen psychotic patients responded with an improvement either in psychosis or depression. Diagnostic categories did not separate the responders from the nonresponders. Only good prognosis, according to Abrams and Taylor's criteria, tended to predict who would respond and who would not. Only a few remitted patients showed postpsychotic decreased depression. We are now studying biochemical and other biological variables to determine which of these may predict a response to lithium. Two papers have been submitted for publication. Dr. Alexander presented this data at the 1977 American Psychiatric Association Annual Meeting.

We examined serum calcium (Ca) and magnesium (Mg) levels in patients before and during neuroleptic treatment (23 patients). Low normal Ca and Mg was an indication of emergence of EPS. Differences in Ca and Mg in drug-free schizophrenic patients were observed between diagnosis and clinical states. Two papers have been submitted for publication and some data were presented at the 1977 World Psychiatric Association Meeting by Dr. Alexander.

D. Gamma-Aminobutyric Acid and the Dopamine Hypothesis of Schizophrenia

(a) A hypothesis has been developed about an ineffective feedback system regulating dopamine in psychosis. γ-Aminobutyric acid (GABA) seems to be implicated in this role. A review paper has been published in the American Journal of Psychiatry.

(b) Diazepam, one of the first compounds found to increase GABA function in the brain, was tried on our patients. We administered diazepam in doses higher than 40 mg per day to five patients. Only two severely psychotic patients received 300 mg of diazepam with equivocal results. One patient, who was still relatively nonpsychotic following pimozide withdrawal, became acutely psychotic when he was withdrawn from 70 mg of diazepam. The more psychotic the patient, the less he was bothered by side-effects like ataxia and sedation.

(c) GABA levels in CSF of schizophrenic patients are presently under study in collaboration with Drs. Post, Bunney and Pert.

E. Endocrinological Project

(a) Evaluation of baseline hormonal levels in schizophrenic patients is being conducted, focusing on hormones that are under total or partial control of dopamine. Prolactin, Growth Hormone, FSH, and LH are measured in serum. Measurements have thus far been made with placebo, pimozide and lithium. Increase in prolactin levels follows dopamine blockade (by pimozide) as expected. A paper was presented at the 1977 New Clinical Drug Evaluation Unit Meeting in Key Biscayne, Florida. A report will be published in the Psychopharmacology Bulletin.

(b) We also examined and studied the growth hormone and prolactin response to apomorphine in drug-free psychotic patients. Patients participated three days after neuroleptic withdrawal after three weeks placebo and one day after GHB withdrawal. This data is presently under analysis.

(c) Age of onset of schizophrenia and sex hormones - A review of the literature in connection with dopamine metabolism has been started and was presented at the 1977 World Psychiatric Association by Dr. Alexander.

F. Cerebrospinal Fluid Studies (CSF) in Schizophrenia

Baseline and probenecid accumulated metabolites of dopamine, norepinephrine, serotonin, tyramine and phenethelamine in CSF are being measured with mass spectrograph.

In 12 patients HVA was evaluated twice during their stay in drug-free conditions--initially, when they were psychotic and four months later when they had improved. During their second drug-free lumbar puncture, HVA appeared to be decreased as was the psychosis.

Evaluation of pimozide effect upon HVA showed that eight responders had a subsequent rise of HVA but three nonresponders had a decrease.

Measurement of viral antibody titers in CSF has been temporarily discontinued.

CSF studies for c-AMP and c-GMP - Mean values were not different from depressed comparison groups of patients. Probenecid increased the baseline values. The effects of pimozide are presently under study.

CSF electrolytes. Dr. Jimerson submitted a paper showing that remitted schizophrenic patients had higher CSF Ca than when psychotic.

G. Dopamine Receptor Blockade Studies

Dr. Rosenblatt started ^{3}H-DA receptor and ^{3}H-spiroperidol receptor studies to measure dopamine blockade in serum of patients on neuroleptics (e.g., pimozide). The collection and assay of the serum is presently under way.

H. Monoamine Oxidase (MAO)

Monoamine oxidase (MAO) in plasma or platelets has been implicated in vulnerability to chronic unremitted schizophrenia and affective disorders. We are presently evaluating clinical and biochemical variables to activity levels. Preliminary data in our male drug-free remitted schizophrenic patients indicate lower than the mean compared to a normal control population. A brief communication has been submitted for publication.

I. Norepinephrine

(a) DBH and norepinephrine are measured in the serum of our drug-free patients and while they are on lithium and on pimozide. Pimozide nor lithium seem to affect circulating serum levels.

(b) DBH in CSF data of 11 schizophrenic patients show similar levels.

(c) Dr. Tallman measured β-norepinephrine receptors on leucocytes from psychotic and nonpsychotic patients. There appears to be no difference between schizophrenic and normal controls while the receptor count stays stable over time. A paper is in preparation.

J. Enkephalins

(a) A hypothesis that endorphins (e.g., enkephalins) may be involved in schizophrenia. A study was undertaken to evaluate a potential therapeutic effect of an opiate antagonist, naloxone, in psychosis. The study was done in collaboration with the Laboratory of Clinical Psychopharmacology, Special Mental Health Research at St. Elizabeths Hospital with equivocal results.

(b) In collaboration with Drs. C. Pert, Post, and Bunney we are measuring enkephalin CSF levels in drug-free psychotic and remitted schizophrenic patients and after pimozide. Data are presently being analyzed.

Major Findings: Psychophysiological

A. Laterality Hypothesis

The Section has continued to test the "laterality" hypothesis. This represents a novel approach to the biology of psychosis. The hypothesis is based on the demonstrated cognitive specialization in man of the cerebral hemispheres. We speculate that in psychosis there is an inappropriate intrusion of right-sided processes. To develop some relevant measures, 16 normals have been tested for differential lateralized cerebral response under a variety of stimulus conditions. A paper has been submitted for publication. These results will be compared with other clinical, biochemical, and pharmacological measures.

B. EEG Recording of Sleep

Sleep studies in relationship to amphetamine and pimozide have been undertaken. Pimozide appears to prevent the amphetamine-induced REM suppression.

C. Psychophysiological Testing

After an interruption of many months because of changes in his laboratory, Dr. Zahn of the Laboratory of Psychology and Psychopathology has started to test our patients again.

Major Findings: Behavioral and Psychological

A. States of Acute Psychosis

A review of the literature has indicated a surprising consistency among the very few and scattered papers which have undertaken a detailed examination of the process of onset and recompensation from acute psychosis. We prepared a review of this area illustrating the applicability of the schema with abstracts from reports of our own patients. Additionally, a rating scale allows the nursing staff to make daily assessment of eight stages which seem best to describe phenomenally each individual patient each day. This has proven extremely easy for the nursing staff to apply and is yielding very high interrater reliability. This observation appears to be of great importance in clarifying the biological correlates of psychotic illness and allowing for the sophisticated differentiation of state- versus trait-phenomena in schizophrenia. A paper has been accepted by the American Journal of Psychiatry for publication. Dr. Docherty will present reliability and validity data at the 1977 World Psychiatric Association Meeting.

B. Psychotherapy and Pharmacotherapy: Conceptual Issues

C. Information-processing Project

(a) A simply administered, objective, quantified measure of cognitive disorder has been developed. Preliminary results have disclosed a highly significant difference between patients and normals. We will use this technique to investigate the effects of psychoactive drugs and state variables on schizophrenic thought disorder. We are also investigating the presence and degree of thought disorder in the families of schizophrenic patients which appeared to be shared by at least one parent in all our studied patients. A paper has been submitted for publication.

(b) Schizophrenic thought disorder is also being investigated with a battery of psychological tests mainly derived from standard I.Q. tests; some scores appear to be impaired in schizophrenia. This emphasizes the need for a special psychological evaluation report.

Major Findings: Genetic

A. HL-A Typing Acute Schizophrenia

HL-A antigens are genetically determined human cell membrane antigens found on a variety of cell types. The genes controlling their expression are found on chromosome 6 and are thought to be linked closely to genes control-

ling immune responses to a number of antigens. In recent years associations of certain HL-A tyes and certain diseases have been found. These diseases are frequently those with associated or causal disorders of immunity, possibly the result of aberrant immune-response genes--thus their association with HL-A.

Schizophrenia may have causal or secondary immune abnormalities; therefore, a study of HL-A antigen frequencies in this disease seemed worthwhile. If found, this would constitute more evidence for the role of the immune system in schizophrenia. To date we have typed 50 patients with schizophrenia.

B. Colorblindness

To find a possible marker for schizophrenia, patients are routinely checked for colorblindness. The colorblindness gene is localized on the X chromosome and has been linked in some families with manic-depressive illness. At this point, no colorblind patients have been found in our group.

Significance to Mental Health Research

These studies contribute to the understanding of a complex psychiatric disorder from which two million Americans are suffering. Our work integrates the multidisciplinary approaches of this disorder.

Pimozide attenuated the amphetamine-induced sleep changes indicating a role for dopamine in the observed sleep disorder. This may have implications for narcolepsy, a disorder in which amphetamine increases wakefulness. Amphetamine raised norepinephrine acutely as well as blood pressure and pulse, effects that were not affected by lithium or pimozide.

We have shown that pimozide is an antipsychotic and that it raises prolactin.

Schizophrenic patients have problems with organizing the information input compared to normals.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00081-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Heritable Characteristics of Cation Transport in Primary Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	E. S. Gershon, M.D.	Chief, Unit on Psychogenetics	BPB NIMH
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0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal area of focus for this project is the identification of heritable characteristics of ion transport which may distinguish individuals with a primary affective disorder from normal controls. The present characteristic under study is the distribution of lithium ions across the membrane of the human erythrocytes in in vivo conditions. A study of twenty-five patients with major affective illness found that the intraerythrocyte to plasma (RBC/plasma) ratio of lithium ion concentration did not discriminate between unipolar and bipolar patients with major affective illness. A racial difference, with black patients having a higher ratio than white patients, was confirmed.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: To determine heritable characteristics of ion transport which distinguish individuals with a primary affective disorder from normal controls.

Methods Employed: The in vivo accumulation of lithium ion by erythrocytes has previously been reported to be under genetic control to discriminate bipolar patients from unipolar patients and from controls. We have studied this ratio to determine if it would serve as a genetic marker of the tendency to bipolar affective illness. The first requirement for a genetic marker is that it distinguish the patients with the illness from controls.

Past and present inpatients of the research wards of the NIMH with an unequivocal diagnosis of primary affective disorder were selected for studies.

In vivo measurement of intraerythrocyte lithium ion was performed after a mean duration of four years of lithium treatment.

The reliability of the erythrocytes/plasma lithium ratio was studied by observing twenty-one of the twenty-five patients two times at one month intervals. The overall correlation between the RBC/plasma ratios between the two visits was 0.87; using the paired t statistic no significant difference was observed.

A correlation of 0.41 was observed between the daily dose and the mean RBC/plasma lithium ion concentration ratio for each individual. The RBC/plasma ratio did not distinguish bipolar I patients ($0.45 \pm .03$, $n = 17$) from the unipolar patients ($0.42 \pm .05$, $n = 4$). The three black patients were excluded from this analysis on the basis of racial differences in a previous report. Their mean ratio in the present study was .6, which was significantly higher than the white patients.

Course of Project and Significance to Mental Health Research and the Program of the Institute: The basis of lithium carbonate's efficacy in the treatment of primary affective disorders is not established. Recent hypotheses of possible membrane abnormalities manifested by differences in ion transport have been advanced. The present study fails to confirm that differences in the equilibrium ratio of intra to extra cellular lithium are a distinguishing characteristic of bipolar patients. Since these results were prepared, the physiologic mechanism responsible for the ratio has been identified as a counterflow exchange system. Under physiologic conditions lithium is not present and this system operates to exchange sodium ions between the intra and extra cellular spaces, without an energy expenditure. We are currently considering whether this system, which can be studied directly by the use of phlo[illegible]in, should be studied in our patients.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00082-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Life Events, Social Support and Alienation in Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to measure the relationship between the number and kind of life events, the amount of subjectively felt social support and social alienation to mood disorders and to changes in specified biochemical levels in affectively disturbed patients vs. controls. The Inventory of Life Events and Support scale, adapted from earlier research scales, will be used in conjunction with the family study interview and blood studies of patients and controls and their family members.

Project Description:

Objective:

1. To develop a standardized rating scale of live events, social support and alienation which can be used to collect data about these psychological factors in order to compare them with biological findings.

2. To evaluate the life events in a sample of 100 patients with mood disorders and their relatives and to compare these findings with those from matched controls.

3. To determine (a) whether the number and kind of life events are the same or different in the patient and nonpatient populations, (b) whether there is an accumulation of events for patients vs. nonpatients, (c) whether there is a family culture associated with more life events regardless of whether these were resident or nonresident family members in contrast to the controls.

4. To correlate the number and stress of life events and alienation with the biochemical levels of DBH, COMT, MAO, etc. which will be obtained under a separate project from this same sample.

5. To evaluate whether the amount of social support in addition to life events is different between patient and nonpatient populations.

6. To assess whether the patient population is more socially alienated than the nonpatient.

7. To evaluate whether the biological findings are different in the same patient in relation to the kind and number of life events at different times.

Methods Employed: Data on life events, social support and social alienation will be collected by a scale adapted from the work of Cochrane (The Life Events Inventory), Nuckolls (Psychosocial Assets Life Crisis and Prognosis of Pregnancy), and Srole's Anomie Scale.

The Inventory of Life Events and Support (ILES) will be incorporated as part of the family social data interview being conducted with this sample of patients and controls. The scale measures the kind and number of events, the perceived stress of those events, opinions about anomie measures, and the degree of involvement with social contacts. Assuming the events chosen for the scale may not be exhaustive of those events happening to people, provision is made for the respondents to supply others.

With a sub-sample, those who are successive inpatient admissions, the scale will be administered during their hospitalization and again one year after their discharge for a comparison of findings. This test-retest method may answer questions such as whether patients have a raised rate of events only prior to illness or over their lives, but leading to illness only some of the time; or whether there are different kinds of events which lead to illness.

Factor or cluster analysis and analysis of variance methods will be used to analyze the data.

Progress to date: During the reporting year approximately 300 scales have been completed by probands and their relatives. During the last half of the year the scale has been completed by subjects in the New Haven, Conn. community to provide control data.

Significance to Mental Health Research: The meaning of life events in mood disorders has been under investigation for some time. Although this present research will replicate the earlier work, it also provides new features. Data about life events of both patients and their relatives (resident and non-resident) will be gathered to ascertain whether there is a family culture of events in contrast to those of nonpatient controls and their families. It will also measure the amount of social support the individual feels from his environment and reflect on whether this makes them better able to handle events. It also intends to measure the association between social alienation and mood disorders. This research will analyze the findings on life events and stress with other family data and a number of biological findings. To our knowledge these investigations have not been done before.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00083-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Hypothesis of X-linkage in Bipolar and Unipolar Affective Disorder

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. Mendlewicz, Ph. D.	Institut de Psychiatrie	Brussels, Belgium
	O. Rafaelsen, Ph. D.	Psychochemistry Institute	Copenhagen, Denmark

COOPERATING UNITS (if any)
Yale University; Harvard University; Blood Bank, Clinical Center, NIH
World Health Organization centers for collaborative study

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical genetic models of X-chromosome transmission and studies of genetic linkage to X-chromosome markers are being performed in primary affective disorders. Two new mathematical models for analysis of two generation data and linkage studies have been developed. The first takes penetrance and variable age of onset into account and also gives more precise opportunities for ascertainment analysis. The second takes into account familial non-X-chromosome illness as a source of heterogeneity in the data, and represents a new theoretical development. Family study data and data on Xg blood group antigen and color blindness markers of the X-chromosome are currently being collected. Neither Xg nor color blindness is linked to bipolar affective disorder in our preliminary data analysis.

Other Investigators Continued:

H. Van Praag, Ph. D.	Univ. of Groningen Groningen, Netherlands
M. Vartanian, Ph. D.	Academy of Med. Sciences, Moscow, U.S.S.R.
J. Avni, M.D.	Hadassah Univ. Hospital, Jerusalem, Israel
P. Bech, M.D.	Psychochemistry Institute Copenhagen, Denmark
H. Beckmann, M.D.	Munich, West Germany
M. Gastpar, M.D.	Psychiatrische Clinic Univ. Basel Basel, Switzerland
R. Rosenberg, M.D.	Psychochemistry Institute Copenhagen, Denmark

Project Description:

Objectives:

1. To develop new data pertinent to the hypothesis that some affective disorders are transmitted as a single gene on the X-chromosome. This hypothesis was given great impetus by the studies of Mendlewicz, Fieve and Fleiss, in which linkage to both the Xga blood group and protan and deutan color blindness was reported.

2. To develop and test appropriate mathematical models of X-linked inh (- tance in psychiatric illness, and apply them to existing and newly collected data.

Methods Employed:

1. Patients with Bipolar or Unipolar affective disorder and their first degree relatives (parents, sibs, children) are studied for psychiatric status, color blindness and for Xg blood type. If it is determined that a family is genetically informative for Xg or color blindness, more distant relatives are studied until a large pedigree with multiple informative sibships is obtained. Analysis is performed using the method of lod scores and using multigeneration models.

2. Mathematical models have been developed for liability to a psychiatric disorder, with multiple thresholds according to sex and form of disorder found (bipolar, unipolar, or related disorders.) These are then applied for goodness-of-fit to existing and currently collected data on prevalences in relatives.

3. New methods of genetic analyses are being developed because psychia (c illness is different from classical genetic markers, in the multiple manifestations of disorder, variable age of onset, penetrance, heterogeneity of disease entities and methods of case ascertainment.

Major Findings:

1. The search for families informative for Xg and color blindness is proceeding. Twenty-one families in which color blindness and major affective disorders are present have been found. Of these fourteen had bipolar probands. Seven of these families are informative for the question of linkage and four additional families appear as if they may be informative on further investigation and examination of possibly heterozygous women. Two families were uninformative because of male-to-male transmission of affective illness, or color blindness occurring in a relative who could not transmit affective illness according to the hypothesis of X-chromosome transmission. One family was uninformative because a heterozygous woman has children who are less than twenty years of age.

Fifteen families informative for the hypothesis of Xg linkage have been identified, and an additional five families are still being investigated and may prove to be informative.

Preliminary analysis of data collected so far does not suggest that linkage is present, but it must be emphasized that the final analysis requires application of complex genetic models so that conclusions should not be drawn at this point.

2. Large pedigree studies of families with X-chromosome markers are continuing. The largest informative family examined to date has had fifty persons seen and evaluated psychiatrically and for genetic markers.

3. New methods of mathematical analysis of genetic data have been developed in collaboration with Dr. Steve Matthysse of Harvard University. The first method allows for correct analysis of linkage data when cases are ascertained through doubly ill probands (color-blind, manic depressive males). This had been the case in previous studies and tends to be the case in all studies because of the greater availability of patients and their relatives.

A method for analysis of linkage in the presence of variable age of onset and reduced penetrance has been developed. This method has recently been programmed for the NIH DCRT computer, and a paper for publication will be prepared.

Lastly, a new analytic approach has been developed which allows for heterogeneity in the genetic etiology of the observed disorder. This represents a new departure and most closely approximates the hypothesis that some bipolar affective disorder may be linked to X-chromosome markers but that there are other genetic causes of the illness. Further work on this model of linkage in the presence of biologic heterogeneity will be performed during the coming year.

4. A collaborative study of linkage of color blindness to manic depressive illness is proceeding under the sponsorship of the World Health Organization (WHO). Two meetings were held in Copenhagen this year. At the first meeting, held in October 1976 the chairmen of the collaborating centers met and agreed on the goals of the projects. At the second meeting, held in March 1977, the

persons actually performing the investigations met and standardized diagnostic terms and clinical interviewing procedures among themselves. This allows for greater opportunity for reliability across centers, which is a fundamental requirement for cross national studies. Informative families are being searched for in Moscow, Basel, Munich, Copenhagen, Brussels and Jerusalem, in addition to the NIMH intramural patients.

Proposed Course of Project:

Data Collection: The NIMH data collection will proceed until 100 bipolar I patients and their relatives have been studied. To this time 78 bipolar I patients, 22 bipolar II patients, and 40 unipolar patients and their relatives have been studied. A great deal of effort is needed after the initial phase of data collection to be sure that all relatives who may be informative for the linkage hypothesis have been seen.

Mathematical model development will proceed as outlined above. The analytic models are expected to be ready before the data collection is complete.

Significance of These Findings: The attempt to separate out an X-linked form of affective disorder is a most important and powerful investigative strategy. If the hypothesis were confirmed, it would constitute proof of a genetic factor in the mood disorders. It would also allow more complete definition of the biological and psychological effects of an "affective disorders genotype" than has been possible until now. For these reasons, this hypothesis must be subjected to the most rigorous possible tests and methods of analysis.

It appears that a definitive answer to the question of linkage will result from our project and from the WHO collaborative study. The findings so far are negative, suggesting that linkage to the region of the Xg locus is not present for bipolar affective disorder.

Publications:

1. Gershon, E.S., Belmaker, R.H., Kety, S.S. and Rosenbaum, M. (Editors): Impact of biology on modern psychiatry. Plenum Press, N.Y. 1977.

2. Gershon, E.S.: Genetic and Biologic Studies of Affective Illness. In Impact of biology on modern psychiatry, Gershon, E.S., Belmaker, R.H., Kety, S.S. and Rosenbaum, M. (Eds.) Plenum Press, N.Y. pp. 207-228, 1977.

3. Gershon, E.S., Bunney, W.E. Jr.: The question of X-linkage in bipolar manic-depressive illness. J. Psychiat. Res., 13:99-117, 1976.

4. Gershon, E.S., Matthysse, S.: X-linkage: Ascertainment through doubly ill probands. J. Psychiat. Res. In press.

5. Gershon, E.S.: The search for genetic markers in affective disorders. In Proceedings of American College of Neuropsychopharmacology. Raven Press, New York, in press.

6. Leckman, J.F., and Gershon, E.S.: Autosomal models of sex effect in bipolar-related major affective illness. Journal of Psychiatric Research. Accepted for publication.

7. Gershon, E.S., Targum, S.D., Kessler, L.R., Mazure, C.M. and Bunney, W.E. Jr.: Genetic studies and biologic strategies in the affective disorders. Progress in Medical Genetics, In press.

8. Gershon, E.S., Bunney, W.E. Jr., Leckman, J.F., VanEerdewegh, M. and Debauche, B.A.: The inheritance of affective disorders: a review of the data and hypotheses. Beh. Genet. Vol. 6, No. 3, 227-261, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00084-03 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Genetic-Biologic Studies of Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	F. K. Goodwin, M.D.	Chief	CPB NIMH
	L. Cytryn, M.D.	Medical Officer, Research	BPB NIMH
	R. O. Rieder, M.D.	Research Psychiatrist	LPP NIMH
	M. M. Weissman, Ph. D.	Associate Professor	Yale Univ.
	K. Kidd, Ph. D.	Assistant Professor	Yale Univ.
	B. Knobe, Ph. D.	---	Intermetrics Corp.
	K. E. Knobe, B.S.	---	Boston University
	R. L. Belmaker, M.D.	Jerusalem Mental Health Center-Ezrath Nashim	
	R. J. Wyatt, M.D.	Chief, Clin. Psychopharmacology	SMRC NIMH

COOPERATING UNITS (if any)
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Division of Medical Genetics, UCLA School of Medicine; CNB, NIMH
Yale University

LAB/BRANCH
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3.7	2.1	1.6

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A family study of primary affective disorders is being performed. Children at risk within these families are studied for predictors of vulnerability to adult disorders. Biologic characteristics of the patients are evaluated for usefulness as genetic markers of vulnerability to affective disorders. Among the biologic variables studied in this way are platelet monoamine oxidase activity and erythrocyte catechol-O-methyl transferase activity. Genetic linkage of affective disorders to known chromosomal markers is also studied. Mathematical models of genetic transmission are being developed for application to these data. Methodological developments are underway in assessment of diagnostic reliability and in development of a data banking system for clinical research.

Other Investigators Continued:

S. Zalcman, M.D.	Clinical Associate	SMRC NIMH
D. McKnew, M.D.	Guest Worker	BPB NIMH
M. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BPB NIMH
J. F. Leckman, M.D.	Resident in Psychiatry	Yale Univ.
E. D. Dibble, D.S.W.	Social Worker	BPB NIMH
N. Rogentine, M.D.	Senior Investigator	NCI
R. S. Sparkes, M.D.	Chief, Div. Medical Genetics	UCLA
M. A. Spence, Ph. D.	Associate Professor	UCLA
D. L. Murphy, M.D.	Chief	CNB NIMH
W. Sceery, M.S.W., J.D.	HSO Director	BPB NIMH
S. D. Targum, M.D.	Clinical Associate	BPB NIMH
L. R. Kessler, M.D.	Medical Officer	BPB NIMH
C. M. Mazure, M.S.	Psychologist	BPB NIMH

Project Description:

Objectives:

1. To identify markers of genetically transmitted vulnerability to affective disorders, where the marker is implicated in the pathophysiology of the disorders.

2. To identify linkage between affective disorders and known chromosomal linkage markers. Such markers may be physiologically unrelated to mood disorders.

3. To develop and apply mathematical models and methods of analysis for genetics of psychiatric disorders and of quantitative variables. Psychiatric disorders present formidable problems for such analyses, because of variable penetrance and age of onset, and because of multiple manifestations of single disorders.

4. Methodological development: A. to develop reliable methods of psychiatric diagnosis by direct examination and by family history. B. to develop a data banking system for clinical research which is compatible with the needs of genetic studies.

Methods Employed: Processes which have been implicated in the pathophysiology of affective disorders, and are being studied in our protocols, include: (1) enzymes active in the synthesis and catabolism of catecholamines, particularly erythrocyte catechol-O-methyl transferase (COMT), and platelet monoamine oxidase (MAO); (2) cortical evoked potential amplitudes in relation to stimulus intensity. (3) cation transport; (4) chromosomal linkage markers are also studied in patients and family members. These include color blindness, Xg blood group, G6PD (in Black or Mediterranean ancestry families), 6-P-Gluconate Dehydrogenase, Acid Phosphatase, Haptoglobin and group Specific Component, HLA antigens, and phenylalanine hydroxylase deficiency.

Major Findings:

1. Identification of Etiologic Genetic Markers in Affective Disorders

We have recently developed a new method for demonstrating that a biologic finding is part of the genetic etiology of an illness. This method is extremely robust in that it is valid for virtually any mode of genetic transmission and is applicable when there is reduced penetrance. Our method is perhaps best presented by example - the hypothesis that decreased platelet MAO is associated with bipolar illness was supported in the preliminary data reported by us on 32 bipolar patients. Examination of one hundred relatives of these patients revealed that on the average both ill and well relatives had lower platelet MAO activity than controls. These findings do not test whether reduced platelet MAO activity is required for transmission of the illness or is simply a marker of a vulnerable segment of the population. To test this we restricted our inquiry to the relatives of those patients who had reduced platelet MAO (since we have no hypothesis about the cause of bipolar illness in patients without this finding). For relatives of low MAO patients, those relatives with unequivocally normal or elevated monoamine oxidase activity showed no psychiatric disorder, whereas 35% of the 17 relatives with markedly reduced platelet MAO activity had major or minor affective disorder. This suggests that decreased platelet MAO is a genetic factor in the transmission of bipolar illness, albeit with reduced penetrance. Further data will be collected prior to publication of this finding, since the current analytic method requires relatively large amounts of data to reach statistical significance.

2. Methodological Developments

Reliability. The key to sound genetic family and epidemiologic studies of psychiatric illness is valid and reproducible diagnostic methods. What is needed is a method of obtaining a lifetime history of psychiatric diagnosis that is reproducible over time and that can be derived from a single patient contact. We have demonstrated two types of reliability in diagnosis: the reliability of simultaneous observation of the same person, and the stability over time of those observations. Using systematic diagnostic criteria and a structured past history interview, we examined 47 cases consisting of patients' relatives and controls. Diagnostic agreement of tests and re-test diagnosis was found in 40 out of 47 cases, with an overall kappa statistic of .79 indicating that this is a high level of reliability. Eighteen out of 21 persons with major affective disorders on any interview were consistently diagnosed. The unreliability of diagnosis came in distinguishing people with minor affective disorders from controls. The simultaneous coding reliability was demonstrated to be very nearly perfect.

A data bank system has been developed which allows access to designated types of relatives according to the observed characteristics of the patient (such as age, sex or biological marker status) and of the relative. This system has advantages of great flexibility, particularly in not requiring uniformity of data elements collected for each person included in the bank. It also can handle very complicated information requests which arise frequently

in analyzing family data. It is more appropriate for clinical research needs than the data bank programs supplied by computer manufacturers (such as the IRS system supplied by IBM) since the latter are designed for manipulation of much larger and more uniform data bases. Furthermore our system operates in batch rather than on-line modes and so offers a significant economic advantage.

3. Family and Pedigree Studies of Affective Disorders

For testing hypothesis of mode of genetic transmission and linkage to chromosomal markers we are conducting a large family study of affective disorders with direct examination and collection of biologic data from large numbers of relatives. To date we have examined 78 bipolar I patients, 22 bipolar II patients, 43 unipolar patients, 798 relatives and 372 controls. Five hundred forty-three of these interviews were performed between July 76 and June 77.

A collaborative family study of affective disorders is on-going between the Section on Psychogenetics and the Depression Research Unit headed by Dr. Myrna M. Weissman at Yale University, where Drs. Weissman and Kenneth K. Kidd are the collaborators. We have carefully standardized interview and diagnostic methods with these collaborators. They will collect data on 50 hospitalized unipolar patients, 100 non-hospitalized unipolar patients and 100 controls, and four first-degree relatives of each of the 250 probands plus additional relatives who are of interest.

We anticipate that at the time that this study is completed it will represent the largest controlled family study of mood disorders performed to date, and will enable much more precise testing of genetic and biologic-genetic hypothesis in the affective disorders.

Personality factors in the transmission of affective disorders are being studied using the Marke-Nyman scale and the Maudsley Personality Inventory.

4. Childhood in Families of Patients with Mood Disorders

The prevalence of psychiatric illness during childhood in the offspring of our patients is being studied in collaboration with the Unit on Childhood Mental Health Studies. Children are examined directly by that unit as described in another report (#Z01 MH 00098-02 AP). Preliminary studies indicate that depression is significantly more prevalent in the children whose parents or grandparents have major affective disorders than in control children.

Since the number studied in this way is small, we are also doing a family history study surveying all probands in the NIMH cohort and in the collaborating cohort at Yale where there are children currently less than 18 years of age.

5. Other Genetic Markers

Studies of cortical average evoked response (AER) augmentation revealed that although bipolar patients augment more than controls, and the augmentation

measure is heritable, it is not a marker of individual vulnerability to affective disorders.

Plasma monoamine oxidase activity was found not to distinguish affective disorders from controls, and not to be a genetic marker of vulnerability, although it was demonstrated to be heritable.

Heterozygosity for phenylketonuria has been suggested as a factor in vulnerability to psychiatric disorder. We are planning to test the frequency of heterozygosity among patients in the family study using phenylalanine loading test.

The CNS responsivity to opiate stimulation will be tested using the neuroendocrine response of decreased LH and testosterone after administration of levorphanol.

6. Histocompatibility Lucocite Antigen (HLA)

This set of closely linked genetic markers has been suggested to be associated with several chronic diseases in man. Recent reports of particular HLA alleles being associated with bipolar illness have not been confirmed by us. Although there are no statistically significant associations of HLA types of illness within our data, the tendency for allele BW17 and BW16 to be somewhat increased in frequency among our patients has led us to perform pedigree studies in families informative for this allele. One family in which the allele and the psychiatric illness appear to segregate has been found and further families are being studied.

Proposed Course of the Project: One hundred bipolar I, 50 unipolar and 50 pedigrees with either bipolar II or schizoaffective disorder are to be studied to complete this investigation. During the coming year our major effort will be to complete the study of pedigrees already begun and to apply our recently developed methods of genetic analyses to these data. The estimated duration of the projects described here is approximately one and one-half to two years longer.

Significance of this Research: We are concerned with an unquestionably familial and chronic illness which appears to have manifestations beginning in childhood in many persons. A genetic or other predictive marker of vulnerability, and precise definition of the mechanism of transmission, would be of importance to therapeutic intervention and to further basic research. The suggestion that decreased platelet MAO activity may be a marker of an individual's vulnerability to affective disorder may prove to be of particular significance in this regard.

Publications:

1. Gershon, E.S., and Buchsbaum, M.A.: A genetic study of average evoked response augmenting/reducing in affective disorders. Shagass, C. and Gershon, S. (Editors) In Psychopathology and Brain Dysfunction (American Psychopathological Association, 66th Annual Meeting 1976) pp. 279-290, Raven Press, New York.

2. Gershon, E.S., Belmaker, R. H., Kety, S.S. and Rosenbaum, M. (Editors): Impact of Biology on Modern Psychiatry. Plenum Press, N.Y. 1977.

3. Gershon, E.S., Bunney, W. E., Jr., Leckman, J.F., Van Eerdewegh, M. and Debauche, B. A.: The inheritance of affective disorders: A review of data and hypotheses. Beh. Genet., Vol. 6 No. 3, 227-261, 1976.

4. Gershon, E.S.: Genetic and biologic studies of affective illness. In Impact of Biology on Modern Psychiatry, Gershon, E.S., Belmaker, R.H., Kety, S.S. and Rosenbaum, M. (Editors) Plenum Press, N.Y. pp. 207-228, 1977.

5. Liebowitz, J. H., Rudy, W., Gershon, E.S. and Gillis, A.: A pharmacogenetic case report: Lithium-responsive post-psychotic antisocial behavior. Comprehensive Psychiatry, 17, No. 5: 655-660, 1976.

6. Gershon, E. S., Jonas, W. Z.: Inherited differences of brain and erythrocyte soluble catechol-O-methyl-transferase activity in two mouse strains. Biological Psychiatry, Vol. 11, No. 5, 641-645, 1976.

7. Gershon, E.S., Belmaker, R. H., Ebstein, R., Jonas, W. Z.:Plasma monoamine oxidase activity unrelated to genetic vulnerability to primary affective illness. Arch. Gen. Psychiatry. Vol. 34, No. 6, 731-734, 1977.

8. Gershon, E. S., Matthysse, S.: X-linkage: Ascertainment through doubly ill probands. J. Psychiatr. Res. In Press.

9. Gershon, E.S., Targum, S. D., Kessler, L. R., Mazure, C. M., Bunney, W. E. Jr.: Genetic studies and biologic strategies in the affective disorders. Progress in Medical Genetics, In press.

10. Gershon, E. S.: The search for genetic markers in affective disorders. In Proceedings of American College of Neuropsychopharmacology. Raven Press, New York, In press.

11. Leckman, J. F., Gershon, E. S., Nichols, A. S. and Murphy, D. L.: Reduced platelet monoamine oxidase activity in first degree relatives of individuals with bipolar affective disorders: A preliminary report. Archives of General Psychiatry. 34:601-606, 1977.

12. Leckman, J. F. and Gershon, E. S.: Autosomal models of sex effect in bipolar-related major affective illness. Journal of Psychiatric Research. Accepted for publication.

13. Gershon, E.S., Kessler, L.R., and Bunney, W.E. Jr.: Monoamine Enzymes and Inhibitors in Affective Illness. Proc. of meeting on Biochemistry and Function of Monoamine Enzymes, Steamboat Springs, Colorado, Marcel Dekker, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00085-01 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Pharmacogenetics of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. S. Gershon, M.D.	Chief, Unit on Psychogenetics	BPB NIMH
OTHER:	W. E. Bunney, Jr., M.D.	Chief	BPB NIMH
	M. H. Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS NIMH
	R. Lake, M.D.	Staff Psychiatrist	LCS NIMH
	D. C. Jimerson, M.D.	Staff Psychiatrist	BPB NIMH
	L. R. Kessler, M.D.	Medical Officer	BPB NIMH
	M. S. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BPB NIMH

COOPERATING UNITS (if any)
LCS

LAB/BRANCH
Biological Psychiatry Branch

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Section on Psychogenetics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The inheritance of variability in the behavioral and physiologic effects of psychopharmacologic agents in clinical use will be studied. The initial project will be on pharmacogenetics of d-amphetamine. Heritability and intra-individual stability of pharmacokinetic data and of the following parameters of response will be determined: 1) behavioral ratings 2) adenergic receptor response 3) neuroendocrine response and 4) cortical evoked response.

PHS-6040

Project Description:

Pharmacogenetic studies in psychopharmacology have largely been devoted to inherited differences in drug metabolism as opposed to genetics of physiologic and psychologic responses to drugs. The behavioral pharmacogenetics of psychoactive drugs in particular is an area in which little systematic investigation has been undertaken, although there have been enough clinical reports of patien and relatives treated with the same medication to suggest that further study will be fruitful.

Our first pharmacogenetic protocol is a study of d-amphetamine. Individual differences in response to amphetamine have been used as a predictor of clinical response to tricyclic anti-depressants and as such may be an important clue to the genetic vulnerability to affective disorders. In this project, monozygotic and dizygotic twins and same-sex sibling pairs of volunteers will be studied. Careful examination will exclude all persons with pre-existing psychiatric or medical disorders that are not minor.

d-Amphetamine is an indirect agonist of dopamine and norepinephrine neurons in the central nervous system. There has been much recent interest in the effects of amphetamine and amphetamine-like compounds such as methylphenidate in mania, schizophrenia and depression. In the present protocol, heritability and intra-individual stability of the central nervous system activation and euphoria responses to amphetamine will be studied using behavioral and cortical evoked potential measures. Neuroendocrine measures will provide heritability estimat of another type of central nervous system response. Plasma norepinephrine and other responses will also be studied genetically.

These pharmacogenetic protocols, in addition to furthering our knowledge of the genetics of response to psychoactive drugs, may also provide a basis for comparison in future pharmacogenetic and other studies of the same psychoactive drugs in major psychiatric illness.

Major Findings:

None - protocol not yet executed.

Significance to Mental Health Research and the Program of the Institute:

Genetic factors appear of importance in the etiology of the major psychiatric disorders, schizophrenia and the affective disorders. Genetic factors may also enter into the response to treatment agents, and a fuller understanding of these pharmacogenetic variables offers possible aid in prediction of treatment response clinically and in unmasking the underlying genetically transmitted pat ophysiology of the illness.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00086-01 BP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. S. Gershon, M.D.	Chief, Unit on Psychogenetics	BPB NIMH
OTHER:	L. R. Kessler, M.D.	Medical Officer	BPB NIMH
	C. Mazure, M.D.	Psychologist	BPB NIMH
	E. D. Dibble, D.S.W.	Social Worker	BPB NIMH
	F. K. Goodwin, M.D.	Chief	CPB NIMH
	P. Gold	Chief, Outpatient & Follow-up Unit	CPB NIMH
	Y. B. Davenport, M.S.W.	Social Worker	CPB NIMH
	G. Grover	Clinical Nurse	CC Nursing

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CC Nursing

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1.75	1.25	0.5

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[ ] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient clinic provides a "well state" patient population for genetic and pharmacologic studies of persons with unipolar (major depressive) and bipolar (manic-depressive) disorders. Such a "well state" population is crucial in identifying biochemical, pharmacologic, or physiologic abnormalities which are present in persons vulnerable to affective disorder, and which may serve as genetic markers to affective illness. Studies of lithium transport suggested that the erythrocyte-plasma lithium ratio is not a marker of vulnerability to bipolar illness.

PHS-6040
(Rev. 10-76)

Project Description:

The purpose of the project is to have a "well state" patient population who would be available for participation in the following research areas:

1. Identification of biochemical, pharmacologic, or physiologic abnormalities which are present in persons who have had major affective disorder but who do not currently require hospitalization.

2. Identification of biological markers of a genetically transmitted predisposition to affective disorders.

3. Creation of a pool of available patients and relatives for further protocols which will be developed and submitted on the genetics of affective disorders, and on longitudinal studies of affective disorders.

Patients are admitted to the study upon referral from the wards of the NIMH intramural program or from various community referral resources. Twenty-two patients are currently enrolled in the BPB portion of the outpatient clinic.

It should be noted that the outpatient clinic is a joint project of the BPB and the CPB. A separate report is submitted for each branch.

Identification of biochemical, pharmacologic or physiologic vulnerabilities to affective illness: The strategy in this type of study is to identify a biologic vulnerability to illness which is not state dependent but present or evocable even when the patient is in the "well state" or in remission.

Pharmacologic studies: We found that the red blood cell to plasma ratio of lithium ion concentration failed to distinguish unipolar from bipolar patients. All 25 patients were in clinical remission at the time of the project. This study failed to replicate previous findings of an elevated lithium ratio in patients with bipolar illness, and suggests that this ratio will not serve as a genetic marker in bipolar illness. (see separate project report # Z01 MH 00081-03 BP)

Another project which will be administered in the outpatient clinic is the recently proposed protocol "Response to amphetamine infusion before and after chronic haloperidol treatment in bipolar patients". A demonstration of supersensitivity of catecholamine receptors during a brief amphetamine infusion will be attempted in 20 patients with bipolar manic-depressive illness in remission. Supersensitivity will be induced by two week treatment with clinical doses of haloperidol, a catecholamine receptor blocking agent used in bipolar illness. Supersensitivity of CNS catecholamine receptors has not been previously demonstrated in man, although it has been proposed that this is a mechanism in the vulnerability to bipolar illness. Behavioral, neuroendocrine, biochemical and neurophysiological responses to amphetamine infusion at two times will be compared: (1) prior to haloperidol administration, and (2) after cessation of a two week course of haloperidol, during the time when supersensitivity has been present in basic pharmacologic studies.

Concurrently, the protocol "Pharmacogenetic studies of d-amphetamine in normal volunteers" will be carried out to study the inheritance of variability in the behavioral and physiologic effects of d-amphetamine, and to provide control data. The heritability of behavioral and biologic response will also be determined from the response parameters in members of a sibling or twin pair.

Biological markers of a genetically transmitted predisposition to affective disorders. Linkage to known chromosomal markers is a strategy for demonstrating that a characteristic is inherited and for demonstrating the mode of inheritance. Patients and relatives in this clinic are studied for chromosomal linkage markers, such as blood group phenotype, HLA phenotype and known biochemical polymorphisms, as described in project report # Z01 MH 00084-03 BP.

Clinical studies of the well state in affective disorders: Pre-existing conceptions on familial and genetic questions in affective illness, and expectations of affective illness and its consequences in one's self and relatives, are being studied through questionnaire and clinical interviews. This information may prove useful for genetic counseling in psychiatry, a service for which there is a growing demand. A controlled study of marital adjustment using the KDS-15 questionnaire developed by E. Frank (Univ. of Pittsburgh) will be performed to continue studies of the disordered marital relationships that characterize bipolar affective illness.

Proposed Course of Project: The priority during the past year has been on recruiting patients and on preparing pharmacologic protocols. For the coming year, we will continue to recruit, with a goal of 50 patients with a history of major affective illness under our care. Execution of the first series of pharmacologic protocols described above will have begun by the end of fiscal 1977.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00116-03 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Interactions of Neurotransmitters and Membrane Proteins at Bimolecular Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Kafka	Physiologist	BP NIMH
Other:	R. Blumenthal	Visiting Scientist	LTB NCI

COOPERATING UNITS (if any)

Laboratory of Theoretical Biology, National Cancer Institute

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine-β-hydroxylase, an enzyme catalyzing the conversion of dopamine to norepinephrine, is found in the membranes of adrenal medullary cell chromaffin vesicles. Dopamine-β-hydroxylase interacts with its substrate, dopamine, and calcium at the bimolecular lipid membrane to form channels through which ions pass. As the ion conductance increases linearly with the potential across the membrane, the potential itself does not open or close, i.e., "gate" the channels. To produce a conductance increase, the natural conformation of the enzyme is required, and norepinephrine, the product of the reaction catalyzed by the enzyme, cannot substitute for dopamine, its substrate. The experiments on bimolecular lipid membranes may provide a physical model for a biological action of dopamine-β-hydroxylase. Dopamine-β-hydroxylase in the membrane of the adrenal medullary chromaffin vesicle may combine with its substrate, dopamine, to catalyze the conversion of dopamine to norepinephrine and at the same time increase the permeability of the vesicle membrane to ions, thereby affecting synthesis, storage, or release of the vesicle catecholamines.

Project Description:

Objectives. To study interactions between neurotransmitters, hormones, membrane proteins, and bimolecular lipid membranes (BLM). The bimolecular lipid membranes separating two aqueous compartments may serve as a model for the cell plasma membrane separating interstitial fluid from cytoplasm or a model of an intracellular membrane separating the cytoplasm from a vesicle interior, e.g., a secretory vesicle membrane.

Methods Employed. A BLM is formed so as to separate two aqueous compartments containing buffer and ions. A pair of KCL-calomel electrodes, one in each compartment, is used to apply a potential across the membrane and to conduct current resulting from the potential. At zero applied potential, a diffusion potential across the membrane can be measured. The electrical conductance of the membrane can be calculated from Ohm's Law.

Dopamine-β-hydroxylase, an enzyme catalyzing the conversion of dopamine to norepinephrine is added to the aqueous compartments and allowed to interact with a bimolecular lipid membrane made from oxidized cholesterol.

Major Findings:

(1) Dopamine-β-hydroxylase, added to both sides of the bimolecular lipid membrane in the presence of calcium and dopamine, increases the conductance of ions through the membrane. (2) The conductance increase occurs in discr steps of 0.4 - 0.7 nmho each and is non-selective. (3) The conductance increases as the square of the dopamine-β-hydroxylase concentration. (4) Dopamine-β-hydroxylase, denatured by heat, does not increase the conductance. (5) When norepinephrine replaces dopamine, no conductance increase is observed. (6) The conductance increase is linear with the applied potential. (7) Dopamine-β-hydroxylase, added to only one side of the bimolecular lipid membrane, also increases the electrical conductance in the presence of calcium and dopamine. (8) When dopamine-β-hydroxylase is added to only one side of the membrane, the conductance increases linearly with the dopamine-β-hydroxylase concentration.

Significance to Biomedical Research and the Program of the Institute:

Dopamine-β-hydroxylase, an enzyme catalyzing the conversion of dopamine to norepinephrine, is found in the membranes of adrenal medullary cell chromaffin vesicles. Dopamine-β-hydroxylase interacts with its substrate, dopamine, and calcium at the bimolecular lipid membrane, to form channels through which ions pass. As the ion conductance increases linearly with the potential, t potential itself does not open or close, i.e., "gate," the channels. To produce a conductance increase, the natural conformation of the enzyme is required and norepinephrine, the product of the enzyme reactions, cannot substitute for dopamine.

The experiments on bimolecular lipid membranes may provide a physical model for a biological action of dopamine-β-hydroxylase. Dopamine-β-hydroxylase

in the membrane of the adrenal medullary chromaffin vesicle may combine with its substrate dopamine to catalyze the conversion of dopamine to norepinephrine and at the same time increase the permeability of the vesicle membranes to ions, thereby affecting synthesis, storage, or release of the vesicular catecholamines.

Proposed Course of the Project:

To study additional actions of neurotransmitters and hormones on membranes by studying the interactions of neurotransmitters, hormones, ions, psychoactive drugs with the BLM, employing the electrical measurements discussed here in addition to measurement of isotopic fluxes reported last year.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZOI MH 00117-02 BP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

α- and β-adrenergic and Prostaglandin Receptors in Human Blood Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Kafka	Research Physiologist	BP NIMH
	J. F. Tallman	Head, Section on Biochemistry and Pharmacology	BP NIMH
Other:	C. C. Smith	Chemist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alpha- and β-adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic drugs to activate or inhibit adenylyl cyclase is used as a measure of their function. Binding of tritiated adrenergic receptor antagonists to membrane receptors on the blood cells is being measured. These correlative measures are being used to assess α- and β-adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.

Objectives:

(1) To develop methods to study the physiological function of α- and β-adrenergic receptors and prostaglandin receptors in easily obtainable human tissue. (2) To study alterations in receptor number or function in pathological conditions which are characterized by altered receptor sensitivity and in psychiatric diseases. (3) To provide an experimental model for the study of drug and/or physiologically induced changes in central receptor sensitivity in man. (4) To understand the membrane perturbations or alterations connecting receptors occupied by their agonists with the inhibition or activation of adenylyl cyclases and subsequent physiological events.

Methods Employed:

To measure α-adrenergic receptors, human platelets are prepared from freshly drawn blood. A portion of the platelets is washed and resuspended for measurement of cyclic AMP production. A second portion is homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α-adrenergic antagonist binding. To measure β-adrenergic receptors a leukocyte preparation is made from human blood using a dextran polymer to induce rapid settling of the erythrocytes. The leukocyte-rich plasma is centrifuged at low speed to yield a leukocyte pellet. This pellet is washed several times in 0.9% saline solution and is used to measure binding of a tritiated β-antagonist and production of cAMP.

Cyclic nucleotides are determined after incubation of intact leukocytes or platelets with the drugs under study by a competitive protein binding assay for cAMP or by radioimmunoassay for cGMP.

Major Findings:

α-Adrenergic Function. Human platelets contain α-adrenergic receptors and prostaglandin-stimulable adenylyl cyclase, but not β-adrenergic receptors. Prostaglandin E_1 (PGE_1) stimulates the formation of cAMP in platelets and α-adrenergic agonists inhibit PGE_1-stimulated cAMP production in the order of potency, l-epinephrine $\geq$ l-norepinephrine >> l-isoproterenol. α-Adrenergic agonist inhibition of cAMP production is reversed by the α-adrenergic antagonists phentolamine and dihydroergocryptine. [^{3}H]dihydroergocryptine, an α-adrenergic antagonist, binds to human platelet membranes. Binding is inhibited by α-adrenergic agonists in the order l-epinephrine $\geq$ l-norepinephrine >> l-isoproterenol, and by α-adrenergic antagonists phentolamine, phenoxybenzamine, and dihydroergocryptine, but not by β-adrenergic antagonist propranolol.

α-Adrenergic agonists inhibit cAMP production half-maximally at about the same concentration that they half-maximally inhibit binding of [^{3}H]dihydroergocryptine. The prostaglandin receptor in human platelets is stimulated by prostaglandins in the order: $PGE_1 > PGE_2 > PGF_{1\alpha} > PGF_{2\alpha}$.

β-adrenergic Function. Leukocytes contain β-adrenergic and prostaglandin-stimulable adenylyl cyclases. The β-adrenergic-stimulable cyclase is

particularly enriched in the lymphocyte-monocyte subfraction of leukocytes. A prostaglandin stimulable cyclase is found in this fraction. The β-adrenergic receptor of leukocytes is stimulated by agonists in the following order of potency: l- isoproterenol > l-epinephrine > l-norepinephrine. The prostaglandin receptor is stimulated by individual prostaglandins in the following order: $PGE_1 > PGE_2 > PGF_{1\alpha} > PGF_{2\alpha}$.

Significance to Biomedical Research and the Program of the Institute:

It is anticipated that results obtained from these experiments will be directly applicable to an assessment of receptor functioning in various human disease states and in monitoring the physiological effects of various drug treatments. If the peripheral α- and β-adrenergic receptors are related to or can serve as a model for central nervous system adrenergic receptors, valuable information may be obtained for the first time about the functioning of these central receptors in psychiatric diseases.

Proposed Course:

Further examination of the mechanism of information transfer from α- and β-adrenergic and the prostaglandin receptor to adenylyl cyclase is anticipated. Presently, studies of these receptors are underway in schizophrenic patients, in cycling manic-depressive patients, and other patients receiving various therapeutic regimens.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00118-01 BP

PERIOD COVERED

September 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Induction of β-adrenergic Receptors in HeLa Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Tallman	Head, Section on Biochemistry and Pharmacology	BP NIMH
Other:	R. C. Henneberry	Senior Scientist	LMB NINCDS
	C. C. Smith	Chemist	BP NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [X] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The induction of β-adrenergic receptors in HeLa cells has been studied using 1-[^{3}H]dihydroalprenolol to measure binding to the receptor and 1-isoproterenol activation of adenylate cyclase to measure function. After sodium butyrate treatment, the number of 1-[^{3}H]dihydroalprenolol binding sites but not their affinity increases and the ability of 1-isoproterenol to activate adenylate cyclase in butyrate treated cells is enhanced. The increase in binding sites and their "coupling" to adenylate cyclase requires the synthesis of new protein; the induction of receptors is specific for butyrate as other closely related short-chain fatty acids are less potent inducers. By manipulating butyrate concentrations, it is possible to separate induction of receptors from coupling to adenylate cyclase and study these as independent events.

PHS-6040
(Rev. 10-76)

The presence of β-adrenergic receptors in HeLa cells has been established using 1-[^{3}H]dihydroalprenolol to measure binding to the receptor and l-isoproterenol stimulated cAMP production to measure the ability of the receptors to function. Binding to these receptors is stereoselective, reversible, and rapid enough to account for the known properties of β-adrenergic receptors. The response to the presence of l-isoproterenol is blocked by classical β-adrenergic blockers but not α-adrenergic blocking drugs.

After sodium butyrate treatment, the number of binding sites for 1-[^{3}H]-dihydroalprenolol increases although the affinity of these receptors for 1-[^{3}H]dihyroalprenolol is unchanged. This increase in binding occurs in conjunction with an increase in the ability of the receptors to activate adenylate cyclase and enhance cAMP production in intact cells after treatment with l-isoproterenol. These increases are rapid, and noticeable increases in binding and cyclase activation occur within a few hours; the increase is virtually complete after 24 hours. It is possible to block the increase in both binding and adenylate cyclase activation with cycloheximide, an inhibitor of protein synthesis; Actinomycin D blocks the increase in binding sites but enhances activation of cyclase by β-adrenergic receptors at low concentrations. The effects of butyrate (a four-carbon fatty acid) on HeLa are rather specific as only propionic (C-3) and valeric (C-5) are able to mimic the effects of butyrate.

By manipulating the concentration of butyrate, it is possible to obtain almost complete induction of binding sites at low butyrate concentrations and by increasing the concentration of butyrate to effect coupling of pre-existing receptors to adenylate cyclase. Thus the induction of receptors and coupling to adenylate cyclase seem to exist as separate events.

The effects of various compounds which have known actions on cell membranes and the motility of proteins on the cell surface have been examined either on both processes or by using a design which allows induction of binding sites and coupling to be examined separately. As an example, Concanavalin A inhibited induction of both β-adrenergic binding and coupling to adenylate cyclase by high butyrate concentration while it stimulated the coupling of pre-induced receptors to cyclase. This type of experimental design will allow us to sort out the role of membrane fluidity and "clustering" of receptors in their ability to activate adenylate cyclase.

Significance to Biomedical Research and the Program of the Institute:

The advantages of a model system to study receptor dynamics and function are tremendous compared to studies with animals. As cells in culture which are readily manipulated provide a stable source of cells which respond in a consistent fashion, the effects of drugs at known concentrations at the cell surface may be studied. The examination of drug effects in the model system can lead to predictions of *in vivo* effects of these drugs in patients. It is also possible to study subsensitivity in this model system which may be related to clinical subsensitive states.

Proposed Course:

Research into the β-adrenergic receptor and adenylate cyclase in HeLa cells will continue and among the questions to be examined are:

1. What is the role of quanyl nucleotides in coupling of receptor to adenylate cyclase?

2. What is the mechanism of catecholamine-induced subsensitivity?

3. Is there another "coupler" protein necessary for β-adrenergic receptors to activate adenylate cyclase?

4. What role does membrane fluidity play in cell surface interactions?

5. What consequent events due to the presence of an active β-adrenergic receptor complex occur in HeLa cells?

Publications:

Tallman, J.F.,Smith, C.C. and Henneberry, R.C.: Induction of functional β-adrenergic receptors in HeLa cells. Proc.Natl.Acad.Sci.USA, 74: 873-877, 1977.

Tallman, J.F.,Fishman, P.H. and Henneberry, R.C.: Determination of sialidase activities in HeLa cells using ganglioside specifically labeled in N-acetyl-neuraminic acid. Arch.Biochem.Biophys. 182: in press, 1977.

Henneberry, R.C.,Smith, C.C. and Tallman, J.F.: Relationship between β-adrenergic receptors and adenylate cyclase in HeLa cells. Nature, 268: in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00119-01 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Putative Neurotransmitters and Modulators in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Tallman	Head, Section on Biochemistry and Pharmacology	BP NIMH
Other:	C. B. Pert	Staff Fellow	BP NIMH
	P. Lerner	Chemist	BP NIMH
	C. C. Smith	Chemist	BP NIMH
	J. W. Thomas	Chemist	BP NIMH
	H. Shibuya	Guest Worker	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.4	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methods were developed for the determination of cAMP, cGMP, γ-aminobutyric acid, substance P and endogenous opiate ligands. Using these methods, it is possible to measure these compounds in a variety of tissues and under many physiological conditions. In particular, all of the above compounds have been or are being measured in cerebrospinal fluid from human subjects. With the available cerebrospinal fluid from patients under study in the Sections of Psychobiology and Neuropsychopharmacology, no differences between patients with schizophrenia and affective disorders could be demonstrated for cAMP, cGMP and GABA. Probenecid dramatically increased the levels of cAMP and cGMP while GABA levels were not elevated by probenecid.

PHS-6040

Using radioimmuno, radioreceptor, or radioenzymatic methods it was possible to develop assays for cAMP, cGMP, GABA, substance P and endogenous opiate ligands. cAMP was measured by a protein binding method which utilized protein kinase from bovine adrenal cortex. The cAMP in the sample to be determined competes with radioactive cAMP for a limited number of binding sites on protein kinase. By using varying amounts of unlabeled cAMP, it is possible to construct a standard curve and thus quantitate the amount of cAMP in the sample. Similarly, GABA and endogenous opiate levels were determined by measuring the ability of the sample (or known amount of GABA or endogenous opiate) to displace the radioactive ligand from a brain membrane preparation which binds GABA or opiates specifically and with a high affinity. Substance P and cGMP were measured using a radioimmunoassay with antibodies either prepared by us or purchased.

Table 1 shows the results of our investigations into cyclic nucleotide levels in cerebrospinal fluid. No striking differences between the different groups of patients could be demonstrated for either baseline cyclic nucleotide levels or some commonly used pharmacological agents on cerebrospinal fluid cyclic some commonly used pharmacological agents on cerebrospinal fluid cyclic nucleotides also did not indicate striking changes in cyclic nucleotide levels (Table 2). Our initial study of GABA in cerebrospinal fluid has also been finished and the results are being prepared for publication. Studies on substance P and endogenous opiate ligands are just beginning.

Table 1

Baseline Probenecid-Induced Accumulations of cAMP and cGMP in Different Diagnostic Groups

	Baseline*		Probenecid*	
	cAMP	cGMP	cAMP	cGMP
Depressed	16.9 ±1.6 (n = 27)	1.4 ±0.4 (n = 15)	80.0 ±5.54 (n = 18)	16.4 ±5.1 (n = 10)
Depressed/ Recovered	20.7 ±1.8 (n = 3)	2.6 ±1.6 (n = 3)	86.0 ±6.0 (n = 3)	19.3 ±5.5 (n = 3)
Schizophrenic	14.2 ±1.3 (n = 29)	1.2 ±0.2 (n = 10)	76.3 ±4.2 (n = 23)	8.0 ±2.6 (n = 8)
Schizophrenic/ Recovered	13.3 ±2.1 (n = 10)	1.3 ±0.6 (n = 7)	59.1 ±6.4 (n = 15)	16.3 ±5.1 (n = 7)
Manic	20.1 ±2.9 (n = 8)	1.6 ±0.4 (n = 6)	103.7 ±11.9 (n = 6)	23.5 ±4.8 (n = 4)
Controls	17.0 ±1.5 (n = 27)	3.0 ±0.7 (n = 12)		

*All concentrations represent the mean values ±S.E.M. and are expressed as pico-moles of cyclic nucleotide per ml CSF.

Table 2

Effects of Psychotropic Drugs on Probenecid-induced Accumulations of cAMP and cGMP

	Probenecid*	
	cAMP	cGMP
Lithium	89.0 ±6.5 (n = 11)	16.8 ±3.9 (n = 9)
Tricyclic Antidepressants	93.4 ±30.3 (n = 5)	4.9 ±3.9 (n = 3)
Piribedil	95.3 ±13.6 (n = 4)	18.9 ±6.2 (n = 4)
Pimozide	95.3 ±4.1 (n = 6)	21.0 ±3.0 (n = 7)

*All concentrations represent the mean values ±S.E.M. and are expressed as pico-moles of cyclic nucleotide per ml CSF.

Significance to Biomedical Research and the Program of the Institute:

Measurement of transmitters and modulators of brain function in cerebrospinal fluid may be important for understanding the metabolism and turnover of these compounds in brain. If differences exist between groups of patients, it may be possible to implicate a transmitter or biochemical event as being important in affective disorders or schizophrenia.

Proposed Course:

We expect to continue our studies on substance P and endogenous opiates in the different groups of patients and to finish the GABA study.

Publications:

Smith, C.C.,Tallman, J.F.,Post, R.B.,van Kammen, D.P.,Jimerson, D.C., Brown, G.L.,Brooks, B.R. and Bunney, W.E.Jr.: An examination of baseline and drug-induced levels of cyclic nucleotides in the cerebrospinal fluid of control and psychiatric patients. Life Sci[1], 19: 131-136, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00125-01 BP

PERIOD COVERED

October 1, 1976 to September 1, 1977

TITLE OF PROJECT (80 characters or less)

Chemotactic Peptide Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. B. Pert	Staff Fellow	BP NIMH
	E. Schiffmann	Biochemist	LDBA NIDR

COOPERATING UNITS (if any)

Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Receptors on rabbit neutrophils which mediate chemotaxis have been demonstrated by direct binding of a small peptide. Bacterial invaders make similar peptides and white cells move to the site of inflammation via interactions with this receptor in the first step of the immune reaction.

PHS-6040

Objectives:

The object was to develop methods for demonstrating a hypothetical receptor on rabbit white cells.

Methods Employed:

Direct binding of radiolabeled peptides was developed under conditions that gave the largest difference between formylated (active) and non-formylated (weak or inactive) ligand displacement in analogy with the development of opiate receptor binding where D- and L- opiate isomers were employed.

Major Finding:

Occupation of this receptor by a series of peptides is closely correlated with their potency as chemotactic agents. Interestingly, at low receptor occupancy, white cells begin to migrate towards the site of inflammation. At high receptor occupancy, they dump their lysosomal enzymes near the site of inflammation. These receptors are the "hardiest" ever studied and resist digestion by all degradative enzymes, a characteristic suitable for their function.

Significance to Biomedical Research and the Program of the Institute:

This receptor is not detectable in brain. However, principles of peptide receptor measurement are learned for every new receptor identified. Knowledge from this white cell peptide receptor will aid in future identification of receptors for neuropeptides which mediate brain function including bombesin, substance P, etc.

Proposed Course:

Individual differences in white cell levels of this receptor will be examined in a number of patient groups who suffer from altered immune responsiveness and also psychiatric disorders. Techniques for demonstrating peptide receptors will be vigorously applied in brain in an effort to identify functionally important neuropeptide receptors.

Publications:

Aswanikumar, S.,Corcoran, B.,Schiffman, E.,Day, A.R.,Freer, R.J.,Showell, H.J., Becker, E.L. and Pert, C.B.: Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. Biochem. Biophys. Res. Comm. 74: 810-817, 1977.

Day, A.R.,Radding, J.,Freer, R.J.,Showell, H.J.,Becker, E.L.,Aswanikumar, S., Corcoran, B.,Schiffman, E. and Pert, C.B.: The synthesis and chemotactic activity of an intrinsically labeled, formylated tripeptide. FEBS Letters, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00143-02 BP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Physiological Function of Opiate Receptors and Their Endogenous Ligands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. B. Pert	Staff Fellow	BP NIMH
Other:	D. L. Bowie	Chemist	BP NIMH
	H. Shibuya	Guest Worker	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate receptors are also receptors for naturally occurring peptides called endorphins or enkephalins. Experiments in this laboratory are aimed at clarifying the identity and function of these peptides: Methods for measuring anodynin, a novel opiate peptide in blood, are under study since blood level alterations may accompany various physiological or psychological states. A radioreceptor assay for cerebrospinal levels of endorphins has also been developed. The molecular basis of opiate agonist-antagonist activity is being probed with D-Ala^2-enkephalin, a novel synthetic opiate peptide which retains high affinity for opiate receptors but resists enzymatic degradation.

Objectives:

1. To determine the chemical and environmental stimuli which release anodynin from the pituitary into the blood and cerebrospinal fluid.

2. To understand the molecular basis of the intrinsic activity of opiate receptors.

3. To develop new opiate peptide agonist and antagonist receptor probes by in vitro analysis.

Methods Employed:

--Receptor binding assay of radiolabeled alkaloid and peptide opiates to rat brain mechanisms.

--Radioimmunoassay of enkephalins and β-endorphin with antisera raised against albumin-coupled derivatives.

--Radioreceptor assay of total opiate receptor ligand content of brain or blood.

Major Findings:

1. Substitution of D-Alanine, the smallest unnatural amino acid for glycine results in a peptide that retains high affinity for opiate receptors, yet resists degradation. In fact, any D-amino acid substitution in this criti position produces a family of analgesics whose potency can be predicted from in vitro binding data.

2. Human and rat blood and cerebrospinal fluid contain a low molecular weight peptide (about 1200) which binds to opiate receptors, produces naloxone-reversible behavioral effects in rats, and resists most enzymatic treatments. Since "anodynin" ("a substance which relieves pain or suffering") disappears from rat blood after hypophysectomy, we are studying a new hormone which serves as a ligand for opiate receptors in the gastrointestinal tract, vas deferens, kidney, etc.

3. The effect of sodium in vitro can be used to predict in vivo agonist or antagonist properties of opiate peptides just as this can be done for opiate alkaloids. The N-allyl derivative of D-Ala^2-enkephalin was synthesized by Dr. Gross and shown to possess antagonist activity in vitro and in vivo.

4. A major discrepancy between the values of opiate ligand in various brain regions as ascertained by radioreceptor and radioimmuno assays suggest that a major undescribed opiate ligand exists in brain. It is neither enkephal nor the high molecular weight endorphins, and may in fact be anodynin.

5. A number of strenuous manipulations of living rats (stress, suckling, drug treatment, etc.) has no effect on pitituary levels of total opiate receptor

ligands. Sodium loading, however, causes a marked depletion of endogenous opiates and suggests that anodynin may be the natriuretic hormone sought by kidney physiologists to explain certain observations.

Significance to Biomedical Research and the Program of the Institute:

Opiates produce profound mood-altering, sleep-inducing and analgetic effects when administered to humans. These effects are due to an artificial mimicking of the natural effects of endogenous morphine-like factor(s) on their specific membrane opiate receptors. This new, previously undescribed neuronal and hormonal system, is a likely candidate for regulating mood, sleep, pain suppression, and urinary output--four behavioral effects which exhibit pathological changes in mental illness. Detailed study of the biochemical functioning and brain distribution of "morphinergic neurons" may elucidate their role in normal and abnormal behavioral states.

Proposed Course:

Three of the most interesting questions we are pursuing are:

Are opiate ligand levels in blood or cerebrospinal fluid related to mood?

Since opiate receptors in brain are associated with numerous areas totally unrelated to pain mediation, what are the other functions of opiate ligands and their receptors?

All behavior data suggests that the opiate receptor can assume agonist-subsensitive and antagonist-supersensitive states. Can a biochemical method be developed for demonstrating these subtle changes in receptor function?

Publications:

Pert, C.: The Opiate Receptor. In Bassett, E.G. (Ed.): Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones, and Small Molecules. New York, Raven Press, 1976, pp. 435-450.

Pert, C.,Snyder, S.H. and Kuhar, M.: Opiate Receptor Binding in Intact Animals. In Ford, D.H. and Clouet, D. (Eds.): Tissue Responses to Addictive Drugs. Holliswood, N.Y., Spectrum Press, 1976, pp. 89-101.

Coyle, J.T. and Pert, C.B.: Ontogenetic development of [^{3}H]naloxone binding in rat brain. Neuropharmacology, 15: 555-560, 1976.

Pert, C.B.,Snyder, S.H. and Portoguese, P.S.: Correlation of opiate receptor affinity with analgetic effects of meperidine homologues. J. Med. Chem. 19: 1248-1250, 1976.

LaMott, C.,Pert, C.B. and Snyder, S.H.: Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section. Brain Res. 112: 407-412, 1976.

Chang, J.-K.,Fong, B.T.W.,Pert, A. and Pert, C.B.: Opiate receptor affinities and behavioral effects of enkephalin: structure-activity relationship of ten synthetic peptide analogues. Life Sci. 18: 1473-1481, 1976.

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Pert, C.B.,Pert, A.,Chang, J.-K. and Fong, B.T.W.: [D-Ala^2]-Met-enkephalinamide: a potent, long-lasting synthetic pentapeptide analgesic. Science, 194: 330-332, 1976.

Pert, C.B.,Kuhar, M.J. and Snyder, S.H.: Opiate receptor: audioradiographic localization in rat brain. Proc. Natl. Acad. Sci. USA, 73: 3729-3733, 1976.

Pert, C.B.,Bowie, D.L.,Fong, B.T.W. and Chang, J.-K.: Synthetic Analogues of Met-enkephalin which Resist Enzymatic Destruction. In Kosterlitz, H.W. (Ed.): Opiates and Endogenous Opioid Peptides. Amsterdam, North Holland, 1976, pp. 79-86.

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Pert, C.B. and Gulley, B.L.: The Mechanism of Opiate Agonist and Antagonist Action. In O'Malley, B.W. and Birnbaumer, L. (Eds.): Hormone Receptors, Vol. II: Peptide Hormones. New York, Academic Press, in press.

Pert, C.B.: Opiate receptors and pain pathways. NRP Bull. in press.

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Pert, C.B.: A request for serum samples from psychiatric patients with associated autoimmune disease: is some psychosis caused by an autoimmune response to neurotransmitter receptors? Communications in Psychopharmacology, in press.

Shibuya, H.,Bowie, D.L. and Pert, C.B.: Opiate receptor ligand in cerebrospinal fluid: a simple radioreceptor assay requiring no preliminary purification. Proceedings of the Neuroscience Society, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00147-02 BP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Behavioral and Physiological Effects of Morphine-like Brain Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Pert	Senior Staff Fellow	BP NIMH
	D. W. Gallager	Research Neuropharmacologist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Microinjections of D-Ala^2-met-enkephalin into the periaqueductal gray matter produced profound analgesia which persisted for 2 to 3 hours. Tolerance and addictive liability were also demonstrated for enkephalin. Intraventricular injections of D-Ala^2-met-enkephalin, morphine and β-endorphin produced a biphasic effect on spontaneous motility--initial depression followed by excitation. The depression and excitation were found to be mediated by different brain structures. Naloxone, an opiate antagonist, was found to depress activity. Microiontophoretic applications of horseradish peroxidase to brainstem nuclei indicated that afferents to the raphe magnus originate from the periaqueductal gray matter and surrounding tegmentum. The adjacent reticular nuclei received afferents mainly from within or caudal to the pons-medulla. Foot-shock stress did not alter the released endogenous opiate ligands into monkey cerebrospinal fluid.

PHS-6040
(Rev. 10-76)

Objectives:

Following the isolation and characterization of enkephalins (opiate-like peptides) from brain by Hughes et al. (Nature 258: 577, 1975) it was suggested that these novel brain peptides may serve as novel neurotransmitters or neuroregulators. The objectives of this project were three fold: (1) To extend our analysis of the physiological functions of these new brain peptides, (2) To characterize their pharmacological effects, and (3) To ascertain the brain circuits encoded by enkephalin and the function of these circuits in determining the pharmacological effects of enkephalins and opiate alkyloids.

Psychopharmacology of Enkephalins In Vivo:

Our initial studies with enkephalins indicated their pharmacological effects were relatively transitory, persisting for only 1 to 5 minutes following direct brain infections (Pert, Simantov and Snyder, Brain Res. in press, 1977; Chang, Fong, Pert and Pert, Life Sci. 18: 1473, 1976). This apparent evanescent response was found to be due to the rapid enzymatic degradation of enkephalins in brain by endogenous peptidases (Chang, Fong, Pert and Pert, Life Sci. 18: 1473, 1976). Our laboratory was able to design a novel enzyme resistant opiate peptide by substituting D-amino acids in the number 2 position of met-enkephalin (Pert, Pert, Chang and Fong, Science, 194: 330, 1976). This new class of compounds proved to be a useful tool in defining the psychopharmacological profile of enkephalins in vivo.

Since enkephalins penetrate the brain with great difficulty, rats were implanted with chronic indwelling cannulae guides through which opiates and opiate peptides could be introduced directly to specific brain sites. Injection targets were those regions which had been demonstrated to possess either high enkephalin or opiate receptor levels. Analgesic activity was assessed following injections into the periaqueductal gray matter, an area which we have previously shown to be critical for mediating the analgesic actions of opiates (Pert and Yaksh, Brain Res. 80: 135, 1974). Since opiates are known to exert profound effects on motility and to produce catalepsy, an attempt was made to define, with microinjection techniques, the neuronal circuitry and mechanisms through which these effects are determined.

Analgesic Sites of Action of Opiates in CNS:

Although we, as well as others, have demonstrated that the medial aspects of the mesencephalon mediate some of the analgesic actions of opiates, recent electrophysiological evidence has also implicated the pontine reticular formation as an important analgesic focus. To ascertain whether this brain region may mediate other aspects of opiate analgesia, rats were assessed for antinociceptive effects in the "tail-flick", "flinch-jump" and "hot-plate" tasks following direct injections of morphine into various hindbrain regions. Systemic effects of morphine were also evaluated following lesions of various hindbrain structures.

Neural Circuitry Underlying Opiate Analgesia:

A number of investigators (Basbaum et al., Proc. Natl. Acad. Sci. USA, 73: 4685, 1977; Proudfit and Anderson, Brain Res. 62: 273, 1975) have recently suggested that the direct action of opiates in the periaqueductal gray matter may produce analgesia, at least in part, by activating a descending inhibitory system that is relayed to the primary afferents through the nucleus raphe magnus in the hindbrain. As a prelude to iontophoretic and single-cell recording studies, we studied the afferent projections to this, as well as adjacent hindbrain nuclei using a retrograde tracer technique with microiontophoretically applied horseradish peroxidase.

Physiological Role of Enkephalin in Brain:

Madden et al. (Nature, 265: 358, 1977) have suggested that enkephalin may be released by brain during stress. An attempt was made to evaluate this possibility by assaying for opiate ligands in the cerebrospinal fluid of chronically perfused rhesus monkeys during and following footshock-induced stress. The assumption behind this study is that enkephalin may be involved in a natural defense mechanism against stress and aversive overloading.

Major Findings:

D-Ala^2-met-enkephalin (5-10 µg) was found to produce a profound analgesic response following injection into the periaqueductal gray matter. The effect had an immediate onset and persisted for 2 to 3 hours. Other similar analogs were also found to produce significant and long-lasting analgesic effects following intracerebral injections. β-endorphin and α-enkephalin (longer opiate peptides derived from β-lipotropin) were also found to produce profound analgesia. On a molar basis, D-Ala^2-met-enkephalin (D-Ala) was found to be one-half as potent as morphine while β-endorphin was found to be 2 to 5 times as potent.

Tolerance developed to the analgesic effects of D-Ala following repeated intraventricular injections. Cross-tolerance was found between morphine and D-Ala following intraventricular injections. Naloxone was found to precipitate a withdrawal syndrome after animals had been made tolerant to D-Ala. The above finding illustrates the addictive liability of enkephalins.

Morphine, D-Ala and β-endorphin (25 µg) were all found to produce an initial inhibition of spontaneous motor activity which was followed by hyperactivity. The depressive phase lasted 1 to 2 hours for D-Ala and approximately 3 hours for morphine and β-endorphin. Tissue injections (5 µg bilateral) revealed differential effects of opiates on motility depending on injection site. Injections into the nucleus accumbens produced only hyperactivity without a depressant component, while injections into the periaqueductal gray matter, surrounding tegmentum and pontine reticular formation produced only depression without excitation. Cataleptic effects also appear to be mediated through the medial aspects of the mesencephalon. Injections into the thalamus, amygdala and caudate nucleus were relatively ineffective in modifying motility.

Naloxone (10 µg bilateral) injected into nucleus accumbens produced a transitory depression of activity. Systemic administration of naloxone (0.1 to 10 mg/kg) was also found to produce a dose-dependent depression of activity. These findings indicate that endogenous opiate peptides in brain may play a role in some aspects of motility. In addition, it appears that the differential effects of opiates on activity are determined through different brain structures.

In general, opiate peptides appear to have pharmacological characteristics quite similar to the opiate alkyloids.

Physiological Role of Enkephalins in Brain:

Rhesus monkeys, implanted with spinal catheters, were subjected to foot shock for 2 hours. Enkephalin levels in cerebrospinal fluid were assessed with the radio-receptor assay. Shock-induced stress did not change levels of enkephalin in cerebrospinal fluid. Enkephalin levels were found to fluctuate over a wide range. These fluctuations, however, could not be correlated with any environmental variables.

Analgesic Sites and Mechanism of Action of Opiates:

Morphine (1 to 5 µg) was found to produce a profound analgesic response following injections into the nucleus giganto-cellularis of the pontine reticular formation. Injections into surrounding hindbrain nuclei were not found to be effective. It is important to note that the nucleus giganto-cellularis is an important terminal region of the spino-reticular pain pathway. This finding adds support to our notion that opiates produce analgesia by inhibiting the terminal regions of the spino-reticular pain pathways. Lesions of the raphe magnus were found to partially antagonize the analgesic effects of morphine following direct injections into the periaqueductal gray matter. This effect was only significant on the "tail-flick" task, which measures a spinal nociceptive reflex, and not on the "flinch-jump" or "hot-plate" tasks.

Neural Circuitry Underlying Opiate Analgesia:

Using the horseradish peroxidase tracer technique, we found that afferents to the brainstem raphe nucleus originate primarily from the mesencephalic central gray and the ventral and dorsal tegmentum. The nucleus reticularis pontis caudalis and the nucleus gigantocellularis were found to receive input primarily from structures within or caudal to the pons-medulla. The demonstration of these afferents from the central gray matter lend neuroanatomical support to the recent suggestion that the direct action of opiates in the mesencephalic central gray, may produce analgesia, at least in part, by activating a descending inhibitory system that is relayed to the primary afferents through the raphe magnus.

Significance to Mental Health Research and the Program of the Institute:

Since opiates are among the most potent psychotomimetic and euphorogenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such novel neurohumors may play an important role in affective states. Furthermore, dysfunctions in the neuronal systems encoded by these peptide neurohumors may underlie some of the etiology of affective disorders.

Proposed Course:

1. Further attempts will be made to define environmental and physiological conditions that can modify enkephalin levels in brain or release into cerebrospinal fluid.

2. The euphorogenic properties of enkephalins will be assessed in the intracranial self-stimulation paradigm.

3. Electrophysiological studies, employing iontophoretic and single-cell recording techniques will be initiated to determine the effects of enkephalins at the neuronal level in areas receiving pain input and those which mediate the effects of opiates on motility.

Publications:

Pert, C.B.,Pert, A. and Tallman, J.F.: Isolation of a novel endogenous opiate analgesic from human blood. Proc.Natl.Acad.Sci.USA, 73: 2226-2230, 1977.

Pert, C.B.,Pert, A.,Chang, J-K. and Fong, B.T.W. [D-Ala^2]-met-enkephalin-amide: a potent, long-lasting synthetic pentapeptide analgesic. Science, 194: 330-332, 1976.

Pert, A. Behavioral pharmacology of D-alanine^2-methionine-enkephalin amide and other long-acting opiate peptides. In Kosterlitz, H.W. (Ed.): Opiates and Endogenous Opioid Peptides. Amsterdam, Elsevier/North Holland, 1976, pp. 87-94.

Pert, A. and Sivit, C. A neuroanatomical focus for morphine and enkephalin-induced hypermotility. Nature, 265: 645-647, 1977.

Pert, C.B.,Bowie, D.L.,Pert, A.,Morell, J.L. and Gross, E.: Sodium ion predicts agonist-antagonist quality of opiate peptides. Nature, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00326-04 CN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical neuropharmacology and psychobiology of mania and depression.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief, Clin. Neuropharmacol. Branch	NIMH
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	Carol Hoover	Social Worker	CN NIMH
	Stanley L. Slater	Clinical Associate	CN NIMH
	Steven Lipper	Clinical Associate	CN NIMH
	David Shiling	Clinical Associate	CN NIMH
	Frank Major	Clinical Associate	CN NIMH
	Paul Grof	Visiting Scientist	CN NIMH
	Robin Murray	Guest Worker	CN NIMH
COLLAB:	Monte S. Buchsbaum	Unit Chief	BP NIMH
	Edward F. Donnelly	Psychologist	SMR NIMH
	Herbert Weingartner	Research Psychologist	BP NIMH
	J. Christain Gillin	Research Psychiatrist	BP NIMH

COOPERATING UNITS (if any)

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Clinical Center Rehabilitation

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	5.0	2.5

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [X] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In continuing studies of drugs with antidepressant and antimanic effects, two monoamine oxidase (MAO) inhibiting agents with relatively specific actions in vitro on the two forms of MAO (MAO-A and MAO-B) have both been found to be clinically active antidepressants. Whether both drugs affect individual depressed patients equally and whether the in vitro specificity is maintained in vivo is under study using clinical and psychological assessment procedures, biochemical and neuroendocrine measures in blood and cerebrospinal fluid, as well as electrophysiological procedures. The blockade by lithium carbonate pretreatment of the stimulant effects of d-amphetamine has been found to be accompanied by changes in cortical average evoked responses and in the excretion of the catecholamine metabolite, 3-methoxy, 4-hydroxy phenyl glycol (MHPG). Fenfluramine's antidepressant effects were found to be accompanied by marked reductions in plasma norepinephrine levels. In other studies, psychological test differences between bipolar and unipolar depressed patients reported several years ago were found to be a replicable distinction in a new, larger patient sample. Other depression-related phenomena in verbal learning and information processing and in behavioral features have been objectively characterized.

Project Description:

Objectives: Individuals with mania, depression and related disorders with affective components, including individuals with severe menstrual cycle-related psychopathology, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patient's self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with investigators in the Biological Psychiatry Branch.

Major Findings:

The series of studies outlined below represents work in varied stages of completion, as most clinical studies extend over a period of two to three years.

Evaluation of Clorgyline and Pargyline as Antidepressants and Inhibitors of Monoamine Oxidase (MAO) Types A and B in Man. This study, begun in pilot fashion in the last year, is an attempt to define whether any greater therapeutic benefit (either greater efficacy or fewer side effects) might be obtained from clorgyline compared to pargyline. These two MAO-inhibitors are structurally-similar propargyline compounds, but manifest differential inhibitory potencies on the two forms of MAO in vitro. We have preliminary indications that this specificity is maintained to a significant extent in vivo in man, although the consequences of differential MAO inhibition in regard to biogenic amine metabolism are not yet known. In our initial studies, carried out by Drs. Lipper, Slater and Shiling, clinical antidepressant efficacy has been clearly demonstrated in some patients, and was confirmed by brief drug withdrawal followed by re-initiation of treatment with either pargyline or another MAO-inhibitor. Further studies of blood levels of the drug (by Drs. Campbell and Shiling), MAO activity (by Dr. Murphy), EEG-monitored sleep changes (by Drs. Mendelson

and Gillin), neuroendocrine measures (by Dr. Slater), CSF amine metabolites (by Dr. Major), plasma norepinephrine (by Dr. Lake), octopamine (by Dr. Saavedra), cardiac systolic-ejection time (by Dr. Gottdiener), psychological test changes (by Dr. Donnelly) and a variety of psychophysiologic measures (by Dr. Buchsbaum) are in progess.

Amphetamine-Lithium Interactions in Man. This study (accomplished as part of the evaluation of the capability of one-day amphetamine trials to predict 28-day imipramine antidepressant responses) has yielded additional results not reported earlier. Analysis of the brain catecholamine metabolite 3-methoxy, 4-hydroxy phenyl glycol (MHPG) in urine (by Drs. van Kammen, Beckmann and Goodwin) has provided evidence of an association between MHPG levels and both the magnitude of stimulant response to d-amphetamine and to the blockade of this response by lithium. Similar data, also emphasizing the existence of large differences between individuals in the responsivity to d-amphetamine, emerged from Dr. Buchsbaum's studies of cortical evoked responses. These studies offer further support for the relevance by amphetamine responsivity and its biological and psychological basis to the mode of action of antidepressant and antimanic drugs.

Fenfluramine as a Serotonin-Related Antidepressant Agent in Man. This drug, which is thought to primarily act through serotonergic mechanisms, was found to have moderate antidepressant affects in bipolar depressed patients. In further studies of its mode of action in man, it was found to produce a marked reversible reduction in plasma norepinephrine levels. This effect may contribute to the moderate anti-hypertensive effects of fenfluramine. It also may be indicative of other information obtained in the last several years on the interaction between norepinephrine, serotonin and other neurotransmitter systems in response to changes in any one system. These adaptational responses as part of the whole question of the role of the serotonergic neurotransmitter system were the subject of a major review by Drs. Campbell, Costa and Murphy completed this year.

Other Studies. Additional investigations carried out on our clinical research unit in conjunction with clinical associates and other staff members from our Branch are reported in detail elsewhere by other NIMH Intramural Research Program groups. These include studies of the state of arousal and mood state in depressed individuals on memory functions, for which Dr. Weingartner is the principal investigator. A mood state-related deficit in longer-term verbal learning capacity and a tendency for certain kinds of learned information to become non-retrievable when mood states have markedly changed have been observed. These experiments have been carried out with Dr. Miller from our group.

In studies of personality features in different subgroups of depressed patients, Dr. Donnelly and our group have succeeded in replicating an earlier investigation indicating that bipolar depressed patients have fewer neurotic and hypochondriacal features than unipolar depressed patients. In general, unipolar patients manifest a much higher level of subjective anxiety and distress compared to bipolar patients.

Associations between suicidal ideation and cortical evoked responses have been described by Dr. Buchsbaum, using data from patients with affective disorders hospitalized on our unit. Several reviews or the mode of action of lithium carbonate and on the prediction of psychoactive drug responses have been completed and will be published soon. Other pilot studies on the potential use of various substances (e.g., phenylethylamine) as antidepressants and on behavioral characteristics as predictors of responses to various psychotropic drugs are in progress.

Publications:

Murphy, D.L.: Effects of lithium on catecholamines and other brain neurotransmitters. In Bunney, W.E., Jr., and Murphy, D.L. (Eds.): The Neurobiology of Lithium. Neurosciences Res. Prog. Bull. 14: 165-182, 1976.

Murphy, D.L., Costa, J.L. and Bunney, W.E., Jr.: Mode of action of Li in treating affective disorders--neurobiological considerations. In Bunney, W.E., Jr., and Murphy, D.L. (Eds.): The Neurobiology of Lithium. Neurosciences Res. Prog. Bull. 14: 183-207, 1976.

Henry, G.M., Buchsbaum, M. and Murphy, D.L.: Intravenous L-DOPA plus carbidopa in depressed patients: Average evoked response, learning, and behavioral changes. Psychosom. Med. 38: 95-105, 1976.

Murphy, D.L.: Biological factors in psychiatric disorders. In Heinzelman, R.V. (Ed.): Annual Reports in Medicinal Chemistry. New York, Academic Press, 1976, Chapter 5, pp. 42-50.

Weingartner, H., Hall, B., Murphy, D.L. and Weinstein, W.: Imagery, affective arousal and memory consolidation. Nature 263: 311-312, 1976.

Donnelly, E.F., Murphy, D.L., Waldman, I.N. and Reynolds, T.D.: MMPI differences between unipolar and bipolar depressed subjects: a replication. J. Clin. Psychol. 32: 610-612.

Beckmann, H., van Kammen, D.P., Goodwin, F.K. and Murphy, D.L.: Urinary excretion of 3-methoxy-4-hydroxyphenylglycol in depressed patients: Modifications by amphetamine and lithium. Biol. Psychiatry 11: 377-387, 1976.

Buchsbaum, M.S.. van Kammen, D.P. and Murphy, D.L.: Individual differences in average evoked responses to d- and l-amphetamine with and without lithium carbonate in depressed patients. Psychopharmacology 51: 129-135, 1977.

Weingartner, H., Miller, H. and Murphy, D.L.: Mood-state-dependent retrieval of verbal associations. J. Abnormal Psychol. 86: 276-284, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00327-05 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Marital Conflict of Manic-Depressive Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. F. Hoover	Social Worker	CN NIMH
Other:	R. G. Fitzgerald	Former NIMH Clinical Associate	CN NIMH
	J. J. Bartko	Mathematical Statistician	MB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study compares manic-depressive patients, their spouses, and individuals from the community in terms of marital conflict, as revealed on the Conflict in Marriage Scale (CIMS).

Project Description:

Objectives: Since anger, expressed or unexpressed, has long been seen as an important dimension in manic-depressive illness, it was proposed: (1) to examine self-reported levels of verbally expressed anger and dissension in the marriages of these patients and their spouses, compared to the acknowledged conflict of marital pairs in the community; (2) to attempt a discrimination between manic-depressive subjects, spouses, and community subjects on the basis of response to 67 CIMS items; (3) to compare patients, spouses, and community pairs on dominance in family decision-making and in the resolution of disagreements, and (4) to explore similarity or complementarity of partners on issues of marital conflict and styles of dealing with anger.

Methods: An agree-disagree card sort, the Conflict in Marriage Scale (CIMS), has been administered to 42 manic-depressive patients and their spouses, as well as to 30 comparable marital pairs from the local community. A discriminant function analysis was performed in an effort to discriminate patients, spouses and community individuals on the basis of their responses to 67 items of the CIMS. In addition, subject responses indicating anger were assessed in relation to coping mechanisms reported, to produce a "conflict ratio" for each individual and couple. These ratios were then used for the purpose of comparing levels of verbally-expressed conflict in the marriages.

Major Findings:

1. Manic-depressive patients and their spouses reported significantly more conflict than community "well" pairs.
2. Patients reported significantly higher levels of verbally expressed marital conflict than their spouses did.
3. Based upon their responses to 67 items of the CIMS, patients, spouses, and community individuals could be distinguished at a high level of probability if sex were known in advance.
4. Spouses were reported to make family decisions more often than either manic-depressive patients or same-sexed community individuals.
5. Conflict ratios between husband and wife in manic-depressive marriages showed virtually no correlation, although the conflict ratios of community pairs were significantly correlated. This suggests that, on dimensions of marital conflict, complementarity may be a greater factor than similarity in manic-depressive marriages.
6. Patients were less likely than their spouses to get busy with work and activity as a means of dealing with anger.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00328-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Monoamine Oxidase Activity and Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Elliott Richelson	Assistant Prof., Pharmacology, Mayo Clinic
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.7	2.3

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is evaluating the associations between individual differences in platelet monoamine oxidase activity and human behavior. Biochemical studies of different human, monkey and rodent tissues have added further information that MAO types A and B are separate enzymes. Studies of psychiatric patients using more refined means to characterize individuals have focussed attention on certain features of chronic schizophrenic patients as most closely correlated with decreased MAO activity. Similarly, studies in college student populations have found moderately strong tendencies for certain personality features and psychosocial difficulties to be associated with reduced MAO activity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project investigates the apparent relationships between monoamine oxidase (MAO) activity and behavior. Because individual differences in MAO activity and drug effects on MAO activity are most readily studied in man using blood platelets as sources of the enzyme, the project has had as a primary objective the more complete biochemical characterization of platelet MAO activity in comparison to MAO activity in brain and other tissues, including cell lines from tissue culture preparations. The study of factors which affect platelet MAO activity and which may be relevant to the interpretation of differences in MAO activity found in psychiatric patient subgroups is another primary objective.

Methods Employed:

Tissues obtained for the study of monoamine oxidase characteristics include human and other mammalian platelets, brain, heart, liver and kidney. Dr. Redmond has provided platelets and other tissues from rhesus monkeys and several other non-human primate species. Dr. Richelson has provided tissue culture preparations of C6 rat glial cells and mouse neuroblastoma cells. Dr. Wyatt and Dr. Berger have provided platelet samples from chronic schizophrenic patients, Dr. van Kammen samples from acute schizophrenic patients, Dr. Grof samples from a manic-depressive group in Canada, and Drs. Buchsbaum, Heier and Schooler samples from normals. A large number of studies correlating MAO activity with clinical, biological (e.g., urinary MHPG, CSF metabolites, plasma norepinephrine and DBH) and pharmacological responses are being conducted on the 3-East Unit and collaboratively with Drs. Goodwin, Post, Zeigler, Lake and other NIMH-IR investigators.

Several different MAO assay procedures have been adapted or developed in this laboratory for the platelet MAO studies, including a rapid combined assay for both the platelet and plasma amine oxidases contributed to by Curt Wright, Anna Nichols, and Dr. Jonathan Costa. Column assay procedures for the measurement of MAO activity in platelets and other tissues have been established by Cynthia Donnelly. Dr. Robert Belmaker has successfully developed electrophoresis procedures for the platelet and plasma amine oxidases in Israel, continuing studies begun here. Dr. Kenneth Kirk has synthesized fluorine-substituted tyramines which have some specific inhibitory and substrate functions for the different MAO forms found in platelets and other tissues.

Major Findings:

Biochemical Studies of MAO A and B in Different Tissues. Using clorgyline and deprenyl as preferential inhibitors of MAO Types A and B and also using different substrates (including phenylethylamine, benzylamine, dopamine, norepinephrine, tryptamine, tyramine and serotonin, as well as fluoro-substituted tyramine and serotonin), we have obtained further evidence that these two enzyme subtypes represent genuinely different enzymes and not different sites or transformations of the same enzyme. This work has been accomplished in rat, monkey and human brain, in human platelets and in cell lines of glioma and

neuroblastoma cells grown using tissue culture procedures. Dr. Sally Anderson is conducting a study of the influence of age differences on brain MAO A and B activity in mice.

Platelet MAO in Psychiatric Patient Groups and Normals. In collaborative studies with Drs. Richard Wyatt and Steven Potkin at St. Elizabeth's Hospital, the reduction in platelet MAO activity previously reported in chronic schizophrenic patients has now been found to be especially characteristic of chronic paranoid patients. Some other clinical features of these patients have also been found to be correlated with platelet MAO activity. In joint studies with a group of investigators at Stanford University (Dr. Berger and coworkers) the reduction in platelet MAO in chronic schizophrenic patients was replicated in a Veterans Administration Hospital sample. Chronicity of hospitalization alone was shown not to be associated with MAO differences, as chronically hospitalized patients with other disorders did not manifest reduced platelet MAO activity.

Studies of college students accomplished by Drs. Buchsbaum, Coursey, Haier and Schooler in collaboration with our group suggest that in non-patient populations platelet MAO may be associated with increased psychosocial difficulties in both the students and their family members. In particular, a higher incidence of suicide attempts in family members of low MAO individuals has been found, confirming similar data obtained from psychiatric patient populations. Characteristic personality features also appear to be correlated with platelet MAO activity in these individuals. These studies have been suggested to represent a new "biological high-risk strategy" for the study of biosocial factors in psychiatric disorders.

Studies of Monoamine Oxidase-Inhibiting Drugs. A comparative trial of two drugs with relatively specific inhibitory properties for MAO-A (clorgyline) or MAO-B (pargyline) has been initiated by Drs. Lipper, Slater and other members of our group. Both drugs have been suggested to be effective antidepressants, but have not been studied for possible differences in their clinical and biological effects. Drs. Campbell and Shiling have developed assays for plasma levels of these drugs, and platelet MAO activity is being monitored during treatment. Dr. Campbell is also conducting a chronic treatment study of low doses of those drugs in rats, done in conjunction with Drs. Robinson, Walker and Lovenberg of NHLBI. A review of the behavioral toxicity of MAO-inhibiting antidepressants was recently published from our group.

Publications:

Murphy, D.L., Donnelly, C.H. and Richelson, E.: Substrate and inhibitor-related characteristics of monoamine oxidase in C6 rat glial cells. J. Neurochem. 26: 1231-1235, 1976.

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Murphy, D.L.: Clinical, genetic, hormonal and drug influences on the activity of human platelet monoamine oxidase. In Wolstenholme, G.E.W. and Knight, J. (Eds.): Monoamine Oxidase and Its Inhibition. (Ciba Foundation Symposium 39), Amsterdam, Elsevier, 1976, pp. 341-351.

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Redmond, D.E., Baulu, J., Murphy, D.L., Loriaux, D.L., Ziegler, M.G. and Lake, C.R.: The effects of testosterone on plasma and platelet monoamine oxidase (MAO) and plasma dopamine-β-hydroxylase (DBH) activities in the male rhesus monkey. Psychosom. Med. 38: 315-326, 1976.

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Buchsbaum, M., Coursey, R.D. and Murphy, D.L.: The biochemical high-risk paradigm: Behavioral and familial correlates of low platelet monoamine oxidase activity. Science 194: 339-341, 1976.

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Redmond, D.E., Jr., Baulu, J., Murphy, D.L.: Pre- and post-partum plasma amine oxidase differences in the rhesus monkey (Macaca mulatta). Experientia 32: 1213-1214, 1976.

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Murphy, D.L., Brand, E., Goldman, T., Baker, M., Wright, C., van Kammen, D. and Gordon, E.: Platelet and plasma amine oxidase inhibition and urinary amine excretion changes during phenelzine treatment. J. Nerv. Ment. Dis. 164: 129-134, 1977.

Murphy, D.L., Belmaker, R., Carpenter, W.T. and Wyatt, R.J.: Monoamine oxidase in chronic schizophrenia: Studies of hormonal and other factors affecting enzyme activity. Br. J. Psychiatry 130: 151-158, 1977.

Murphy, D.L., Belmaker, R.H., Buchsbaum, M., Martin, N.F., Ciaranello, R. and Wyatt, R.J.: Biogenic amine-related enzymes and personality variations in normals. Psychol. Med. 7: 149-157, 1977.

Donnelly, C.H. and Murphy, D.L.: Substrate and inhibitor-related characteristics of human platelet monoamine oxidase. Biochem. Pharmacol. 26: 853-858, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00329-02 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Platelets as Models for the Study of Neurotransmitter Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.3	1.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project continues our examination of the regulation of uptake, storage, and release of serotonin and other biogenic amines in human platelets. Plasma-membrane uptake of serotonin is affected little by metabolic poisons or by the presence of cytoplasmic serotonin, but is inhibited completely during cellular secretion. Although vesicular uptake of serotonin may be associated with proton gradients, plasma-membrane uptake of serotonin does not appear to proceed by a similar mechanism. Vesicular nucleotides are quite restricted in motion in intact cells, possibly due to binding with vesicular calcium. Since total vesicular calcium and phosphorus do not change following large alterations in serotonin content, it seems unlikely that all three substances are stored as micellar complex. Platelets appear to be capable of a graded secretory response, rather than an all-or-none type of secretion, which may be determined in part by the frequency with which each cell has been stimulated while in the circulation.

Project Description:

Objectives: The work continues our investigation of the processes regulating the dynamics of uptake, storage, and secretion of biogenic amines by human platelets. Our initial work has indicated that the regulation is more complex than appreciated from studies employing other types of amine-storing tissues. Accordingly, we have attempted to explore first aminergic function in platelets from normal donors, in the anticipation that such studies will provide the framework within which to examine both the effects of psychoactive drugs and possible genetically-related components of affective illness which may alter aminergic function. Because of the relative ease with which platelets can be obtained and manipulated, we have been able to study these cells by employing a number of different techniques, some of which involve collaborative work with investigators outside the NIH campus.

Methods Employed: Platelets are studied employing three general classes of techniques: (1) radiolabel tracer experiments, (2) spectroscopic techniques (electron spin resonance, nuclear magnetic resonance), and (3) electron-microscopic techniques.

(1) Radiolabel tracer experiments. In order to achieve accurate timing of the kinetics of amine fluxes, the formaldehyde-fixative technique developed previously (Costa and Murphy, 1975) is used. In addition, studies here have shown that brief thrombin treatment can release platelet storage vesicles, thus permitting evaluation of the accumulation of radiolabelled amines in cytoplasmic and vesicular storage compartments (Costa, Murphy, & Kafka, 1977). The exchange of amines between the two compartments has been explored, utilizing serotonin, other biogenic amines, and also the ring-fluorinated analogs of various amines synthesized by Dr. Kirk.

(2) Spectroscopic techniques. The electron spin resonance of lipophilic compounds is used to examine the lipid fluidity of platelet membranes before and after treatment with formaldehyde, in order to evaluate the relationship of membrane fluidity to amine uptake (studies performed in collaboration with Dr. Chignell). The nuclear magnetic resonance of phosphorus residues in intact cells is also examined, employing the high-resolution instrument at Bell Laboratories (with the cooperation of Drs. Ugurbil and Shulman). These studies explore the chemical environment of cytoplasmic and of vesicular adenine nucleotides (stored in conjunction with serotonin), the interrelationships between the two pools, and the behavior of the two pools during secretion.

(3) Electron-microscopic techniques. By collaborating with several investigators, it has been possible to extend our study of platelets to the ultrastructural evaluation of single cells under a variety of conditions. Air-dried whole mounts, examined with conventional transmission electron microscopy, are used to evaluate the pattern of release of platelet vesicles following varying degrees of stimulation (in collaboration with Drs. Detwiler and Feinman). Statistical analysis of the changes is performed with the assistance of Dr. Patlak and Ms. Pettigrew. The vesicular content of metal ions and phosphorus has been examined with the electron microprobe X-ray analysis system (with Dr. Tanaka). Since a 10% change in either calcium or phosphorus content can be detected (Costa, Tanaka, Pettigrew, & Cushing, 1977), the technique has been used to evaluate alterations in vesicular composition following alterations in

vesicular serotonin content. Vesicular movement and secretion is studied in individual living platelets by utilizing the hydration chamber developed by Dr. Hui. Electron energy-loss spectroscopy, done in conjunction with Drs. Joy and Maher at Bell Laboratories, permits the ultrastructural localization of fluorinated amines (utilized also in radiolabel tracer studies) inside individual cells.

Major Findings:

Dynamics of Amine Accumulation Across the Plasma Membrane. Utilizing formaldehyde fixative, it has been possible to study the uptake of amines over periods as short as 10 seconds (Costa & Murphy, 1975). The platelet has a high-affinity uptake system which appears to operate at low 5HT concentrations. Other amines, as well as 5HT at high concentrations, appear to enter the cell by a separate pathway. Fluorine substitution in the 4 and 6 positions increases the rate of transport by a factor of almost 5, while complete inhibition of the cell's ability to generate ATP (with antimycin A) inhibits uptake by only about 20% (unpublished observations). Nevertheless, stimulation of the cell to secrete (with either thrombin or the ionophore A23187) acts rapidly to inhibit completely further net uptake of 5HT across the plasma membrane (Costa & Murphy, 1977). Similar processes operating in serotonergic nerves would act to potentiate the action of released 5HT. Because of our ability to investigate the magnitude of both cytoplasmic and vesicular pools of amine (Costa, Murphy, Kafka, 1977), we have been able to show that plasma-membrane uptake can proceed against a concentration gradient as high as 10,000 to 1. Vesicular uptake is, nevertheless, not a prerequisite for plasma-membrane uptake (Costa, Fauci & Wolfe, 1976). In addition, counter-transport appears not to occur.

Dynamics of Amine Accumulation in Platelet Vesicles. A variety of amines in addition to 5HT can enter platelet vesicles (Costa, Murphy, & Reveille, 1977). A large proportion of the dopamine and octopamine entering the cell is sequestered in vesicles, while tyramine and tryptamine appear to remain predominantly in the cytoplasm. Differences in platelet handling of all five amines are also suggested by the fact that the ionophore X537A removes each amine from vesicles at a different rate. Furthermore, the presence of either dopamine or tyramine in both vesicles and cytoplasm fails to alter the rate of 5HT uptake across either plasma or vesicular membranes, again suggesting that each set contains an uptake mechanism uniquely sensitive to 5HT. Since the difluoro analogue of 5HT, which exists largely as a zwitterion at intracellular pH's, enters vesicles much faster than 5HT itself, there appears to be some basis for accepting the current hypothesis that a zwitterionis form of the amine may be the transported species. Maintenance of vesicular stores of amine appears to be critically dependent on the availability of sufficient intracellular levels of ATP.

Platelet Membrane Fluidity. The lipid component of membranes of intact platelets appears to be more mobile than that of other blood cells examined to date. The addition of formaldehyde fixative in concentrations which stop 5HT uptake significantly decreases the fluidity--a phenomenon which may play a role in the effectiveness of formaldehyde in inhibiting 5HT movement.

Platelet Energy Dynamics and pH Gradients Studied In Intact Cells. Intact cells examined by P^{31} NMR show clear phosphorus resources attributable to cytoplasmic nucleotides and inorganic phosphate. Vesicular nucleotides, in contrast, appear to be sufficiently restricted in mobility that their resonances are too broad to be visible in the NMR spectrum. Since the cytoplasmic phosphate resonance shifts in accordance with extracellular pH changes, the platelet does not appear to maintain a pH gradient across its plasma membrane. Thus, the movement of 5HT into the cell is most probably not proton-coupled.

Models For The Cellular Secretory Response to Stimulation. Platelets do not appear to release their vesicles in an all-or-none fashion, but rather in a gradual manner which may be related to the relative frequency with which a given cell has been stimulated while in the circulation. (Costa, Detwiler, Feinman, Murphy, Patlak, & Pettigrew, 1977; Costa & Hui, 1977). Thus the intracellular release of calcium believed to be a prerequisite for secretion does not act simply to induce release of all vesicles. If vesicular release of 5HT from nerves is governed by similar constraints, transmitter release may be graded according to the prior depolorization status of the nerve terminal.

Evaluation of Changes in The Elemental Composition of Vesicles. The calcium and phosphorus in platelet vesicles (Costa, Tanaka, Pettigrew, and Cushing, 1977) do not vary in the elemental ratio or absolute amount following a 95% reduction or a 50% increase in 5HT content. It thus seems unlikely that the three substances are stored as a micellar complex. In addition, the new technique of energy-loss spectroscopy has allowed us to pinpoint the location of the fluorinated analogs of 5HT inside vesicles (Costa, Joy, Maher, Kirk and Hui, 1977), and should permit further evaluation of alterations in storage packet size.

Significance to Biomedical Research and the Program of the Institute: These explorations of the factors regulating aminergic function in human platelets should provide a more accurate basis for interpreting (1) the effects of psychoactive drugs in clinical use and at a testing stage, and (2) aminergic function in neural tissues such as synaptosomes. In addition, improved understanding of the uptake, storage, and secretion of serotonin in cells readily obtainable from a number of individuals may permit evaluation of genetically-related components of affective illness which alter aminergic function.

Publications:

Costa, J.L., Murphy, D.L., and Kafka, M.S.: Demonstration and evaluation of apparent cytoplasmic and vesicular serotonin compartments in human platelets. Biochem. Pharmacol., 26: 517-521, 1977.

Costa, J.L., Tanaka, Y., Pettigrew, K.D., and Cushing, R.J.: Evaluation of the utility of air-dried whole mounts for quantitative electron microprobe studies of platelet dense bodies. J. Histochem. Cytochem., in press.

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Costa, J.L., Detwiler, T.C., Feinman, R.D., Murphy, D.L., Patlak, C.S., and Pettigrew, K.D.: Quantitative evaluation of the loss of human-platelet dense bodies following stimulation by thrombin or A23187. J. Physiol. 264: 297-306, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01631-01 CN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neuroendocrine Studies In Patients With Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D. J. Shiling	Clinical Associate	CN NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies utilize neuroendocrine tests of hypothalamic-pituitary function to increase our understanding of the central nervous system effects of anti-depressant drugs. The effects of apomorphine, L-Dopa, arginine and insulin on plasma prolactin, growth hormone, cortisol, norepinephrine, and dopamine beta hydroxylase were studied in depressed patients during a pretreatment period and again during treatment with lithium carbonate. The effects of L-tryptophan on plasma prolactin, growth hormone, cortisol, norepinephrine and dopamine beta hydroxylase were studied in depressed patients. The effects of two monoamine oxidase inhibitors (MAOI), clorgyline and pargyline on plasma cortisol and prolactin were studied in depressed patients during a pretreatment period and again during treatment with each MAOI. Cortisol levels were not significantly changed by either MAOI. Prolactin levels were significantly elevated by both MAOI's, with every individual studied demonstrating an increase.

Project Description:

Objectives: These studies are designed to establish the effects of antidepressant medications on hypothalamic-pituitary responsivity. Altered levels of circulating hormones have important clinical sequelae in their own right, e.g., the hyperprolactinemia which may result in galactorrhea in patients receiving psychoactive drugs. Perhaps of even greater interest is theaattempt to use neuroendocrine responsivity as an index of receptor sensitivity to biogenic amines in brain centers thought to be more directly related to the expression of emotionality, mood and behavior.

Methods: Depressed patients, having given their informed consent, participate in neuroendocrine testing while drug free and again while taking antidepressant medications. Multiple samples of venous blood are collected and concentrations of circulating hormones are determined using radioimmunoassay techniques.

Major Findings: Moderate but consistent elevations of plasma prolactin were observed during the chronic administration of both clorgyline and pargyline. In every individual tested prolactin levels were elevated by both MAOI's. Plasma cortisol levels were not significantly altered by either MAOI. The lack of change in plasma cortisol concentrations measured in the same samples suggests that non-specific stress effects during drug treatment do not account for our results. These results are, we believe, the first report of MAOI's altering pituitary secretion.

Data from studies on the hypothalamic-pituitary effects of lithium and L-tryptophan are undergoing analysis at the present time.

Significance to Biomedical Research: Biological hypotheses relating to the cause and treatment of both the affective disorders and schizophrenia invoke the concept of alterations in the functional activity of biogenic amines which function as neurotransmitters in different areas of the central nervous system, as well as control the neuroendocrine cells that govern the secretion of pituitary hormones. The demonstration of alterations in pituitary secretion in relation to treatment with psychoactive medication may be informative of the mechanism of action of these drugs and also perhaps provide evidence to further our understanding of the pathophysiology underlying a variety of psychopathologic states.

Proposed Course: Further characterization of the effects of antidepressant medications on neurohormonal secretions is underway. In collaboration with Dr. Wallace Mendelsohn of the Sleep Lab we are assessing the effects of MAOI's on the relationships between sleep stages as recorded on the sleep EEG and anterior pituitary secretion patterns as documented by 20 minute samplings of venous blood over periods of 24 hours.

It has already been established that (1) depressed patients have altered sleep EEG patterns (e.g., decreased REM sleep latency), (2) pituitary secretion is related to sleep stages (e.g. growth hormone secretion accompanies sleep stages

III and IV) and (3) that chronic administration of MAOI's has a profound effect on the sleep EEG (absence of REM sleep periods).
A more precise understanding of the relationships between these phenomena may suggest a neurochemical disturbance in depressed states, the reversal of which may constitute the basis of the therapeutic effects of some anti-depressant agents.

Publications:

Slater, S., de la Vega, C.E., Skyler, J., and Murphy, D.L.: Plasma prolactin stimulation by fenfluramine and amphetamine. Psychopharmacol. Bull. 12: 26-27, 1976.

Slater, S.L., Lipper, S., Shiling, D.J. and Murphy, D.L.: Monoamine oxidase inhibitors elevate plasma prolactin in man. Lancet, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00446-08 CP

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Inpatient Clinical and Psychopharmacological Studies of Manic-depressive Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	

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[X] (a) HUMAN SUBJECTS [X] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of manic-depressive disorders, particularly with regard to the complex interrelationships among clinical phenomenology, diagnosis, biological variables, and pharmacologic response. The studies are longitudinal; drug trials are double-blind. Major foci during the past year include: (1) the study of regularly cycling manic-depressive patients, and the use of drugs to modify cycle length and intensity; (2) the evaluation of tricyclic antidepressants showing relative specificity for a given amine neurotransmitter; (3) the pharmacokinetics of tricyclics; (4) the evaluation of experimental drugs such as pimozide, cyproheptadine, piribedil and L-tryptophan; (5) the use of psychological testing and auditory evoked response to predict drug response.

Project Description: Z01 MH 00446-08 CP

This project description encompasses a wide range of research studies, many of which are continuations of ongoing investigations. In this group of studies the overall objective is a more comprehensive understanding of the pathophysiology of manic-depressive and schizo-affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups (including subgroups which may predict drug response), the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

In regard to clinical methodology, this year has seen a major effort towards a more systematic approach to diagnosis. Prior to admission, patients are screened by two psychiatrists and a social worker. Selection is based on meeting criteria for major, primary affective disorder, as defined by the research diagnostic criteria of Spitzer, et al. (1975). Following admission a comprehensive history of the present and past illness is recorded using the research diagnostic criteria. The use of this diagnostic system (RDC) is especially valuable since it is in use in other major psychobiological research centers; this will make meaningful comparisons between different investigative groups possible for the first time, and could provide the basis for major advances in research. The depressed patients admitted to the unit generally show psychomotor retardation and/or agitation, sleep disturbance, loss of appetite with weight loss, excessive tiredness, loss of interest, and thought patterns dominated by feelings of hopelessness, helplessness, and guilt. There is frequently a past history of depressive and/or manic episodes, and often a family history of mood disorders. Manic patients typically evidence increased psychomotor activity with an increased initiation and rate of speech, a labile mood characterized by either euphoria or irritability, and a cognitive state characterized by expansiveness, grandiosity, and over-confidence; more severely manic patients may show extreme dysphoria, with disorganized psychotic features which may be difficult to distinguish from schizophrenia cross-sectionally. This year has seen a particular emphasis on regularly cycling manic-depressive patients (see Project #Z01 MH 00450-03 CP).

Behavioral data is collected on a longitudinal basis throughout the course of hospitalization using twice daily global ratings by a trained nursing research team; these ratings focus on depression, anxiety, psychosis, anger, and anxiety. Twice a week a Modified form of the Brief Psychiatric Rating Scale (BPRS) is scored for each patient. Information regarding families is obtained through ongoing contact between the social worker and the patient's relatives. Motor activity is recorded continuously as described in Project #Z01 MH 00450-03 CP.

Sleep is recorded at half-hour intervals throughout the night on all patients. In addition, selected patients are studied for sleep parameters utilizing all night EEG sleep recordings in collaboration with the central sleep facility of the DCBR (Dr. Gillin).

All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The use of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

The major areas of study and major findings during the last year can be divided into separate areas:

1. The ongoing effort towards refinement of instruments for behavioral assessment has continued. In collaboration with the other clinical units use of the Brief Psychiatric Rating Scale (BPRS) has been underway, with cross-ward evaluation of interrater reliability and further development of the modified correlations between individual items and/or clusters of items with the global scores for mania, depression and psychosis

2. A new antidepressant drug trial is underway, involving a crossover between Zimelidine and desmethylimipramine, the former having highly selective effects on reuptake in brain serotonin systems, while the latter is highly specific for norepinephrine systems. The results of this crossover trial will be integrated with our amine metabolite findings which suggest the existence of amine specific "subgroups" of patients with major depressive disorder.

The amino acid precursor of serotonin, L-tryptophan, is being tested in combination with lithium. Preliminary data suggest that used in this way, tryptophan has some antidepressant effects, and in high doses may have psychotogenetic effects, particularly in individuals already manic.

A serotonin receptor blocker, cyproheptadine, is being tried as an antidepressant; there is evidence that this drug can decrease the hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) in Cushing's disease (decrease the positive serotonin drive to the HPA) and that this axis may be hyperactive in some forms of depressive illness, particularly unipolar depression with high anxiety and/or psychosis. Results are as yet inconclusive, although one of the two patients treated with the drug to date appeared to have a clearcut antidepressant response.

3. In collaboration with Robert Post, the evaluation of psychotropic drugs in the treatment of acute mania has continued, focusing on the efficacy of pimozide in comparison with a standard reference neuroleptic (chlorpromazine). Experience to date suggest that pimozide is an effective antimanic agent but appears to have a slower onset of action, particularly in the acutely excited and hyperactive patient.

4. Tricyclic levels in plasma and cerebrospinal fluid: In collaboration with Anthony Zavadil and Sandy Markey, we have continued the study of imipramine and desmethylimipramine levels in the plasma and CSF of patients

receiving imipramine as an antidepressant. Findings to date include the following: There is a very high correlation between the levels of both drugs in plasma and in CSF, but the ratio of DMI to IMI is higher in the CSF than in the plasma; this has important implications since we have also found that the effects of imipramine administration on the serotonin metabolite in CSF shows a positive correlation with imipramine levels but no correlation with DMI levels. These findings may have implications for studies which use amine metabolite measures to predict differential antidepressant response to different tricyclic antidepressants. Clinical response to imipramine correlates with IMI levels in CSF and plasma, but not with DMI levels.

Dr. Potter has examined the question of plasma binding of imipramine. Using C^{14} imipramine; he has found that the affinity of the drug for brain tissue is 45 times greater than it is for plasma protein. We have thus questioned the physiological meaning of "binding" measures derived from in vitro tests in which plasma protein binding is compared to buffer, not to brain tissue.

6. In collaboration with Edward Donnelly and Dennis Murphy, several studies of psychological functioning in depressed and manic patients are underway. These studies include the Halstead-Reitan battery, a test of associative capacity thought to be a sensitive indicator of organic dysfunction, the Wechsler Adult Intelligence Scale, and the MMPI. Preliminary results suggest that depressed patients show significant deficits on the Halstead-Reitan; in the case of the bipolar patients this deficit does not entirely return to normal during the immediate in hospital recovery phase. Studies of IQ have indicated normal ranges for manic-depressive patients, but a striking change (averaging 20 points on the full scale) in the same patient going from depression into hypomania. MMPI studies continue to indicate significant differences between unipolar and bipolar patients in a variety of personality parameters. Some MMPI items have been shown to predict subsequent response, both to lithium and to imipramine, with a high rate of accuracy.

7. In collaboration with Monte Buchsbaum of the Section on Cognitive and Perceptual Studies, BPB, we have continued and extended our studies on the cortical evoked potentials in manic-depressive patients. Attention has focused particularly on the use of differences in the auditory evoked response as a predictor variable in antidepressant drug response. The pretreatment amplitude-intensity slope is significantly greater (augmentation) among lithium responders compared to non-responders, while in the case of imipramine, the reverse in found. Differences in AER among unipolar and bipolar depressed patients are now well established, and within each of these populations AER has been shown to be associated with age of onset and the presence of a family history of affective disorder. In addition, we have conducted longitudinal studies of AER in cycling manic-depressive patients and in normal controls and patients under stress (for details of these studies, see project report from the Section on Cognitive and Perceptual Studies, Biological Psychiatry Branch).

We are particularly interested in expanding and improving our clinical descriptive capacities using sophisticated diagnostic and rating instruments to provide reliable data for factor analysis and cluster analysis. It is expected that the application of these more advanced statistical techniques will go far in clarifying the questions about meaningful subgroups in affective disorders.

In drug efficacy evaluation we continue to focus on longitudinal and intensive study of a few drugs, rather than multiple brief trials using large numbers of new drugs.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00447-08 CP

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Cerebrospinal Fluid Amine Metabolites and Amines in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
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	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Robert Post	Chief, Clinical Research Unit	BP/NIMH
	Edna Gordon	Chief, Unit of Clinical Biochem.	LCS/NIMH
	Daniel van Kammen	Chief, Unit on Neuropsychopharm.	BP/NIMH
	Francis Major	Clinical Associate	CN/NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

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[X] (a) HUMAN SUBJECTS [X] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Amine metabolites in cerebrospinal fluid (CSF) are studied in patients to assess neurochemical alterations which may be associated with clinical diagnoses (particularly affective illness, schizophrenia, and alcoholism), biological subgroups, or drug treatment. Probenecid, which inhibits the removal of the metabolites from CSF into the blood, is administered as part of these studies. Sources of variance have been a major focus of study; multivariate statistics are employed to examine these identified sources of variance, including age, sex, activity, and CSF probenecid levels. Fluorometric and mass spectrometric techniques for measuring acid metabolites have been compared. Several studies demonstrate the use of pretreatment CSF metabolite values in predicting drug response to neuroleptics, piribedil, and tricyclic antidepressants. CSF dopamine-beta-hydroxylase levels are similar across diagnostic groups, but show a state-dependent decrease in mania and may be altered by several drugs. Cyclic-AMP levels are similar in mania, depression, and neurological controls. Abstinent alcoholics show decreased 5HIAA levels compared with controls and alcoholics shortly post-intoxication, suggesting interactions between alcoholism and serotonergic activity.

PHS-6040

The Chief, CPB serves as the coordinator for CSF studies in the Division; the Unit on Neurochemistry, CPB provides sample processing and assays (the latter in collaboration with Edna Gordon of the LCS); this Branch also provides central data storage and analysis for CSF results. The major collaborating groups for the CSF studies are the Sections on Psychobiology (Dr. Post) and Neuropharmacology (Dr. van Kammen) of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch (Drs. Murphy and Major).

This project explores the neurochemistry of various psychiatric conditions using clinical methods previously developed in this Branch, and lab techniques developed in the Branch and in the Unit on Clinical Biochemistry, LCS.

The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients by assay of levels of the amines and their major metabolites in the cerebrospinal fluid (CSF) under baseline conditions and following the administration of probenecid. The metabolites under study are 5-hydroxyindoleacetic acid--5HIAA, the major metabolite of serotonin, homovanillic acid--HVA, the major metabolite of dopamine, and 3-methoxy-4-hydroxyphenylglycol acid--MHPG, and 3-methoxy-4-hydroxyphenylacetic acid--VMA, the major and minor metabolites respectively of norepinephrine; in addition, norepinephrine itself is measured in collaboration with Dr. Ray Lake of the LCS.

A central issue has been the reliability of the laboratory techniques for measuring these amine metabolites. Fluorometric values for 5HIAA and HVA were compared with values obtained from the same sample using mass spectroscopy. There is a very high correlation between the two methodologies for both metabolites; however, the absolute values obtained by the two methods on a given sample differ. For HVA, the fluorometric method resulted in values which were consistently lower than corresponding mass spectrometric values. For 5HIAA, the fluorometric values were consistently higher than the mass spectrometric values. Sources of these discrepancies are being explored in collaboration with Edna Gordon of the LCS.

The probenecid technique itself has been a subject of more extensive investigation. Originally, probenecid in doses of 100 mg/kg p.o. was thought to provide essentially complete blockade of acid metabolites from CSF, thus providing a measure of the rate of production and metabolism of the parent amines. Our more recent work, in collaboration with Michael Ebert of the LCS, demonstrates a highly significant positive correlation between CSF levels of probenecid and levels of both 5HIAA and HVA in a group of drug-free depressed patients, accounting for between 10% and 25% of the observed total variance in metabolite levels after probenecid. This implies either that the blockade induced by probenecid is not complete in these patients, or that higher levels of probenecid induce increased acid metabolite production.

In contrast to the finding in depressed patients, no correlation between CSF probenecid levels and acid metabolite levels in schizophrenic

patients was found, suggesting either possible membrane transport differences, or differences in central sensitivity to probenecid among diagnostic subgroups. Patients with primary affective disorder treated with lithium similarly show no correlation of CSF probenecid with acid metabolite levels, in contrast to the high correlation noted in the same patients in the drug-free state, suggesting that lithium may stabilize either the acid membrane transport system, or the CNS neurotransmitter response to administered probenecid. Finally, a variety of psychoactive drugs were noted to lower CSF probenecid levels significantly, a factor which must be taken into account in studies of amine metabolite changes associated with drug treatment.

A portion of the work involves ongoing efforts to explore the merits of different techniques for administering probenecid. Data gathered from this unit and from Michael Ebert's unit indicate that the intravenous route of probenecid administration can be safely employed, promote moderate levels of probenecid in the CSF and result in a reduced incidence of gastro-intestinal side effects. It remains to be seen which method produces a more meaningful measure of central monoaminergic activity.

In collaboration with the other units as noted above, we have continued the exploration of CSF amine metabolites within and between various diagnostic groups (affective illness, schizophrenia, schizoaffective disorders, character disorders and alcoholism) with the emphasis on primary affective illness. As the continued study of CSF amine metabolites in drug-free depressed patients increases the sample size, multivariate techniques become feasible. Contributions of various factors to the total variance in amine metabolite levels can be evaluated and controlled. For example, the previously reported bimodal distribution of 5HIAA levels following probenecid included variance due to sex, CSF probenecid levels, and sex interacting with probenecid levels. If the 5HIAA values are adjusted for sex and probenecid effects, the bimodal distribution becomes more nearly normal.

One can then assess statistically whether raw 5HIAA values (which reflect indirectly the effects of sex and probenecid) or adjusted 5HIAA values (with variance associated with sex and probenecid removed) are more closely associated with variables such as diagnosis or drug response. Adjusted 5HIAA values are somewhat more powerful predictors of antidepressant response to imipramine in unipolar patients, demonstrating the potential utility of such controls on "extraneous" sources of variance. Studies are underway to explore the usefulness of "adjusting" values to remove contributions to variance associated with clinical traits (e.g., age, sex, diagnosis), clinical states (e.g., psychosis, anxiety, depression), biological characteristics (e.g., enzyme activities, temperature), or dietary intake.

In collaboration with the National Naval Medical Center, a study of CSF amine metabolites in alcoholics during withdrawal from alcohol and during abstinence has been completed. Levels of 5HIAA were found to be lower in abstinent alcoholics than in either alcoholics during the immediate

post-intoxication period or individuals with personality disorder not associated with alcoholism. These findings suggest that central serotonin production and metabolism may be decreased in alcoholics in abstinence, and that acute alcohol ingestion may function to increase central serotonin turnover to levels observed in non-alcoholic controls. Alternatively, the increased 5HIAA during the immediate post-intoxication period may be an effect of alcohol withdrawal rather than alcohol itself.

In a second study of alcoholics, disulfiram was found to decrease CSF HVA significantly, while having no effect on CSF 5HIAA; disulfiram's clinical effects may have some relationship to its effects on central neurotransmitters.

Cyclic AMP levels, studied collaboratively with Robert Post, were found to be similar in manic, depressed, and neurologically ill patients, and to be unchanged during treatment with tricyclics, lithium, ECT, or methadone.

Levels of dopamine beta-hydroxylase (DBH) in CSF have been studied in collaboration with Walter Lovenberg and Pauline Lerner of the NHLBI, using a highly sensitive radioenzymatic assay. There appear to be no significant differences among diagnostic subgroups (bipolar, unipolar, schizoaffective, schizophrenic, alcoholic). In addition, CSF DBH is not correlated with CSF VMA or NE. Preliminary data suggests, however, that monoaminer oxidase inhibitors decrease levels of CSF DBH, while pimozide increases levels. Additionally, in bipolar patients, DBH is significantly lower in mania than in either euthymic or depressed states; finally, there may be a subgroup of schizophrenic patients with very low levels of DBH in their CSF.

Studies of norepinephrine (NE) in CSF of patients with affective illness, done collaboratively with Robert Post and Ray Lake, found that these levels are higher in manic patients than in depressed patients or neurological controls, using baseline or probenecid measures. No difference was found between unipolar and bipolar depressed patients. Positive correlations were found among CSF NE, CSF 5HIAA, and anxiety ratings. These data do not support a simple catecholamine hypothesis postulating reduced central noradrenergic activity in depression as compared with controls, but do suggest an association between increased central noradrenergic turnover and mania.

Drug response prediction using CSF metabolites continues to be a major area of interest. Antidepressant response to piribedil, a dopamine receptor agonist studied in collaboration with Robert Post, shows a strong negative correlation with pre-treatment HVA. This finding suggests that low pre-treatment HVA reflects decreased central dopaminergic activity. The clinical improvement observed relates to the reversal of this decreased activity of the dopamine agonist. Treatment with piribedil decreases CSF HVA, consistent with a negative feedback mechanism regulating the dopaminergic system.

Research in collaboration with Daniel van Kammen's schizophrenia studies group demonstrate a positive association between pretreatment CSF HVA and subsequent response to dopamine receptor blockers (neuroleptics) in schizophrenic patients.

Further research is underway to explore the usefulness of CSF 5HIAA levels in predicting responses to tricyclic antidepressants. Our results suggest that unipolar patients with low 5HIAA levels are more likely to respond to amitriptyline than those with high CSF 5HIAA, consistent with the observations that amitriptyline is a drug with relatively serotonergic effects. The opposite pattern seems to hold for imipramine, a relatively more noradrenergic drug. Attempts are in progress to combine this prediction data with predictions based on urinary norepinephrine metabolites, and to expand these metabolite studies to the prediction of lithium response.

Regarding drug effects on amine metabolites, the imipramine-induced decrease in 5HIAA levels in CSF was found to correlate with CSF imipramine levels, but not with CSF desmethylimipramine, confirming findings reported in animal studies.

A further developing area of study involves relationships between CSF metabolites and neuroendocrine findings. Pituitary thyrotropin response to thyrotropin releasing hormone was negatively correlated with CSF 5HIAA in unipolar patients, while urinary free cortisol was positively correlated with 5HIAA in the same group, suggesting associations among neurotransmitter systems, neuroendocrine systems, and subgroups of affective disorder.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00448-04 CP

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Urinary Norepinephrine Metabolites in Affective Disorders and Normal Controls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Robert Post	Chief, Clinical Research Unit	CP/NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This project evaluates a wide variety of factors which contribute to the urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), the norepinephrine metabolite thought best to reflect brain turnover. Major findings are as follows: (1) 24-hour urinary MHPG excretion is low in depression and high in mania but can not be used to indicate mood change; (2) Depressed patients vs. volunteers show an altered circadian rhythm in MHPG with a maximum difference in the late evening; (3) MHPG changes induced by diet and physical activity are confirmed to be restricted to depressed patients, suggesting defective "buffering" of norepinephrine turnover; (4) urinary free cortisol (UFC), which is controlled by ACTH secretion, is found to have an inverse relationship to urinary MHPG in depressed patients--since ACTH secretion is inhibited by norepinephrine, the findings support the hypothesis that urinary MHPG and UFC are indices of central norepinephrine turnover.

Project Description: Z01 MH 00448-04 CP

The purpose of this continuing project is an extensive study of norepinephrine metabolites (particularly 3-methoxy-4-hydroxyphenylglycol--MHPG) in patients and normal volunteers. MHPG is the norepinephrine metabolite thought best to reflect brain norepinephrine turnover--current clinical studies by Ebert and Kopin in the LCS using radioisotopes to study turnover indicate that a large proportion of MHPG appearing in the urine originates from brain pools of norepinephrine.

Our focus during the last year has been on a more detailed investigation of factors which influence MHPG excretion, especially activity and diet. In order to use MHPG excretion as a tool in evaluating the hypothesis of altered norepinephrine function in depression and mania, the influence of such non-specific factors on MHPG excretion must be understood. State-dependent changes in MHPG excretion may be of even greater importance as suggested by our earlier longitudinal studies in patients with rapid cycles.

The methods for urinary MHPG studies in patients and normal volunteers have been described in the description for this project covering the period July 1, 1975-June 30, 1976. In brief, daily urine samples are collected either in 3-hour segments or as 24-hour pools, measured, refrigerated with preservative, aliquoted, frozen and analyzed for MHPG. Six areas of study may be summarized:

1. Longitudinal studies in two manic-depressive patients with rapid cycles have been followed by studies in five patients in whom one complete cycle with depression and mania have been investigated. Results are consistent with earlier impressions, such that a) there is a trend which requires only a few more subjects to reach significance for urinary MHPG to be higher in the manic phase than in the depressed phase; b) the pattern of fluctuation with change of mood is not precise enough to allow urinary MHPG alteration to be a predictor of switches into mania or depression as had been suggested.

2. Routine application of the patient activity monitor (PAM), a non-telemetric computer-based motor activity monitor, has recently been possible. With this device, the clear rhythm in urinary MHPG already reported for normal volunteers has been shown to follow their regular pattern of activity. Although not originally demonstrable, a similar circadian rhythm in MHPG associated with the level of activity has been found in depressed patients. A striking finding is the difference in the timing of the MHPG circadian rhythm in volunteers vs. depressed patients. The volunteer (control) group has a much later peak than depressed patients so that the maximum difference between the two groups is found in the late evening period. Depressed patients consistently show a lower urinary MHPG than our controls in collections taken at this time. Future studies which focus on a difference in urinary MHPG between depressed patients and controls will greatly benefit from application of this knowledge.

3. Further investigation of effects of diets confirms our finding that depressed patients but not normal volunteers will have a doubled

urinary MHPG excretion when switched from a low to a high monoamine diet. Taken with our previous finding that strenuous physical exercise increased MHPG in depressed patients but not volunteers, we are cautious in the interpretation of data comparing volunteers and patients. In patients, as suggested in our earlier report, we must consider the possibility of a deficiency in "buffering" central and/or peripheral norepinephrine systems against a variety of influences.

4. Other tests of the depressed (or manic) patient's ability to "buffer" neurohumoral systems are now underway. These involve a complex series of neuroendocrine challenge tests during mania and depression, the results of which will be compared to MHPG urinary excretion. For neuroendocrine systems believed to be strongly influenced by norepinephrine we would expect to find either positive or inverse relationships. For instance, ACTH secretion is inhibited by norepinephrine. An acceptable peripheral index of ACTH secretion is now available using a reliable radioimmunoassay for urinary free cortisol. As one would predict we are currently finding an inverse relationship such that as urinary MHPG increases, urinary free cortisol decreases and vice versa.

5. As part of our regular assessment of quantitative methodology, we have given particular attention to the measurement of urinary MHPG. We find a 1.0 rank-order correlation of MHPG determined by us on a GC using an electron capture detector with that determined in collaboration with Edna Gordon of the LCS on a GC-MS. The absolute values do differ, those obtained by GC-MS being 0.8 of those obtained by GC. Although this absolute difference does not affect our relative between-group findings we have initiated further collaborative studies to verify our methodology. A visit to Dekirmenjian's laboratory in Chicago where the GC methodology was first developed with Maas suggests that our current GC method is as reliable and far more efficient than those used elsewhere. We are exchanging samples with Dekirmenjian and will begin to do so with Maas in order to cross-validate the assays.

6. A current study which relates primarily to state dependency of urinary MHPG but will be expanded to include at least some neuroendocrine measures involves "recovered" patients. Either former ward or active clinic patients who are not cycling and who appear euthymic are being admitted for brief periods to the ward for various tests and urine collections. These "well-state" studies of urinary MHPG excretion should allow us to assess whether patients regain their capacity to "buffer" norepinephrine systems. Our findings will help to decide whether urinary MHPG excretion under various easily manipulated conditions (activity, diet, neuroendocrine tests) may be used as a biochemical marker of manic-depressive or major affective illness.

Publications: ZO1 MH 00448-04 CP

Beckmann, H., van Kammen, D.P., Goodwin, F.K. and Murphy, D.L.: Urinary Excretion of 3-Methoxy-4-hydroxyphenylglycol in Depressed Patients: Modifications by Amphetamine and Lithium. Biol. Psychiat. 11:377-387, 1976.

Ebert, M.H., Beckmann, H., Post, R.M. and Goodwin, F.K.: Effect of Moderate Exercise on Urinary MHPG in Depressed Patients. Arch. Gen. Psychiat., in press.

Goodwin, F.K. and Potter, W.Z.: The Biology of Affective Illness: Amine Neurotransmitters and Drug Response. In Cole, Frazier and Schatzberg (Eds.): Depression: Biology, Dynamics and Treatment. New York, Plenum Press, in press.

Muscettola, G., Wehr, T. and Goodwin, F.K.: Effect of Diet on Urinary MHPG Excretion in Depressed Patients and in Normal Controls. Am. J. Psychiat., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00450-03 CP

PERIOD COVERED

July 1, 1976 to Sept 30, 1977

TITLE OF PROJECT (80 characters or less)

Biological Rhythms and Melatonin Studies in Manic-depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
OTHER:	Frederick Goodwin	Chief	CP/NIMH
	Alfred Lewy	Clinical Associate	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Theodore Colburn	Chief, Section on Technical Devel.	NIMH
	Bruce Smith	Staff Fellow, Sec. on Tech. Devel.	NIMH
	Howard Hoffman	Statistician, Biometry Branch	NICHD
	J. Christian Gillin	Chief, Unit on Sleep Studies	BP/NIMH

COOPERATING UNITS (if any)

Section on Technical Development, NIMH; Unit on Sleep Studies, BP/NIMH; GC-MS Facility, LCS/NIMH; Primate Facility, DCBR/NIMH

LAB/BRANCH

Clinical Psychobiology Branch

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INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) These studies focus on a longitudinal analysis of behavioral changes in affective illness, interrelating these observations with physiological and biological variables; emphasis is on circadian (24-hour) and longer rhythms. Results to date indicate that some regularly cycling bipolar patients show circadian desynchronization in temperature and motor activity, so that the normal rhythm is no longer "locked in" to the environmental 24-hour day-night cycle, but tends to drift in and out of phase. Alterations in this cycle can be manipulated by such procedures as day-night reversal and artificial 21-hour days. A new ambulatory non-telemetric physiological monitor has been developed and tested in inpatients and outpatients. The relationship between biological rhythms and melatonin levels can now be explored thanks to the development of a highly sensitive assay based on mass spectrometry. An immunoassay for melatonin has also been developed.

Project Description: Z01 MH 00450-03 CP

Epidemiological evidence has emphasized the essential cyclicity of both unipolar and bipolar affective illness. Since manic-depressive illness is a periodic illness, a study of the role of biological rhythms in its pathophysiology and pathogenesis is important. This project involves longitudinal studies of behavioral, physiological, and biochemical variables in inpatients and outpatients who have relatively frequent mood cycles. Focus is on both circadian (i.e., 24-hour) and longer rhythms, requiring both intensive data collection (multiple sampling within the same day) and longitudinal data collection over time (in several patients, we have obtained daily measures over many months).

Behavioral parameters under study involve both nurses and self-ratings, as well as motor activity monitoring using a nontelemetric solid state, computer-based device developed at NIH. Physiological parameters include EEG sleep studies, temperature, biochemical variables including urinary 3-methoxy-4-hydroxyphenylglycol (MHPG--the urinary metabolite thought best to reflect brain norepinephrine metabolism), melatonin and (less frequently) CSF amine metabolites. These data are subject to spectral analysis in order to characterize circadian and longer rhythms.

Findings in these studies can be described under several headings:

1. We have identified a group of bipolar cycling patients who show circadian rhythm desynchronization in temperature and in motor activity; that is, these circadian rhythms are not locked into phase with the environmental day-night cycle, but tend to drift gradually in and out of phase. In this respect these patients behave as if they had been cut off from all environmental light and time cues; these findings suggest some disconnectedness in the systems responsible for integrating environmental cues into the regulation of biological rhythms. The systems involved in this integration include hypothalamic clock mechanisms, the pineal gland and connecting central norepinephrine pathways.

2. Diurnal patterns of urinary MHPG excretion are under investigation utilizing 3-hour urine collection periods around the clock both in depressed patients and normal controls. Evidence to date indicates a diurnal pattern for urinary MHPG in normal controls with a maximum in the daytime and a minimum at night. Depressed patients as a group show an alteration in timing of diurnal MHPG variation (an earlier peak) as well as amount (lower mean). Compared to controls, depressed patients show decreases in MHPG which are maximal in the late evening. This finding is consistent with the hypothesis that diminished central noradrenergic activity is responsible for abnormal cortisol secretion and decreased REM sleep latency in the late evening reported to exist in depressed patients.

3. The activity-rest cycle is driven by a hypothalamic "pacemaker" in animals and has been the focus of intensive investigation in basic biological rhythm research. Recurrent depressive and manic-depressive illness may be conceptualized as disorders of this activity-rest cycle

"pacemaker" inasmuch as activity monitoring has revealed: a) consistent state-dependent change in activity; b) changes in timing (phase shifts or frequency modulation) of the daily onset of activity; c) "splitting" of the daily activity phase into two components in stable hypomania; and d) state-dependent changes in the ratio of the length of the daily activity phase to the length of the daily rest phase in various patients studied longitudinally to date.

Each of these findings can be induced experimentally in animals with environmental (light, schedules), chemical (endocrine, drug) and anatomical (hypothalamic lesions) manipulations.

In collaboration with T. Colburn and his colleagues at the Technical Development Section, we are greatly expanding our longitudinal activity studies in manic-depressive and recurrent depressive patients, cyclothymics, and normal controls in order to extend our findings of disordered regulation of the activity-rest cycle in patients, and to explore the possible existence of long-range cycles of activation in normals. We are currently also adapting a system for automated longitudinal activity monitoring of animals for use in developing animal models of the cyclic activity changes seen in mood disorders and for investigating effects of antidepressants on biological oscillators.

4. The effects of psychoactive drugs on biological rhythms in manic-depressive patients is of special interest. Lithium has been shown to alter the frequency of free-running circadian rhythms in animals and we are currently evaluating the effects of this drug on biological rhythms in manic-depressive patients with particular attention to possible effects on the nightly rise in thyroid stimulating hormone (TSH), since lithium is known to have potent effects on the hypothalamic-pituitary-thyroid axis.

5. Studies of the effects of weak entraining agents on free-running circadian rhythms in animals provide an experimental paradigm for the induction of long-range cyclic variation in levels of motor activity (the most clearcut "bipolar" feature of manic-depressive illness) complete with recurring "switch-over points." By scheduling patient activities to a 21-hour "day" it is posssible to establish an analogous experimental condition on an open ward. In four bipolar patients studied to date it has been possible in this way to dissociate the activity rest cycle from the circadian temperature rhythm which then free-runs at period lengths greater than 24-hours. The four patients all showed mood changes from depression into mild to marked hypomania midway through the experimental rhythm desynchronization. Using a patient room which has been specially adapted for daytime sleep it has been possible to conduct a type of rhythm experiment heretofore only possible in isolation in underground caves. Future 21-hour day studies will include EEG sleep monitoring; existing studies indicate that percentage REM and REM latency can be systematically altered by transmeridinal flight. Changes in these parameters would be "cyclic" on a 21-hour day and would be

of interest in view of numerous studies showing state-dependent changes in these variables associated with cyclic mood disorders.

6. Our efforts to characterize circadian rhythm alterations in affective disorders now include studies of integrated concentrations of blood constituents by means of a recently developed ambulatory blood pump which continuously draws small amounts of blood from an indwelling catheter. Integrated sampling is a methodological advance which reduces the number of samples necessary to characterize circadian rhythms and reduces variability in the concentrations measured. The device is currently being used in studies of circadian neuroendocrine profiles of depressed and cycling patients and studies of melatonin levels in depressed patients. Four patients have been studied in both mania and depression to date, and samples will be assayed for GH, FSH, LG, TSH, PL and cortisol by S. Yen at the UCSD. The same device will be used to characterize the neuroendocrine and melatonin effects of sleep deprivation, a transient but effective treatment for endogenous depression.

7. Working in Dr. Markey's lab, Dr. Lewy has developed a gas chromatographic mass spectroscopic (GC-MS) assay for melatonin. This assay is highly sensitive and specific, well beyond any previously achieved assays for melatonin. Dr. Lewy has also developed a radioimmunoassay for melatonin and is using the GC-MS as a reference to evaluate the specificity of the immunoassay. Melatonin, produced by the pineal gland, is intimately involved in the regulation of circadian rhythms and reproductive physiology in animals. For example, estrus is inhibited by melatonin; also, locomotor activity in birds is synchronized by melatonin. Melatonin has a nycthemeral rhythm, daylight supressing its synthesis. In women, melatonin peaks during menstruation and falls during ovulation. Finnish women have greater conception and multiple-birth rates during the summer (long) days. Previous work from our group has confirmed its psychoactive properties in man; related clinical work shows it to be an effective blocker of serotonergically mediated neuroendocrine responses. Our studies focus on the question of melatonin regulation by light in normals and patients, on the effects of sleep (dark?) deprivation on melatonin and on the pharmacological effects of drugs currently used in our clinical protocols.

Melatonin is thought to be an indicator, if not a mediator, of a central "biological clock". Many neuroendocrine substances appear to have either nyctohemeral or diurnal rhythms. Melatonin may regulate diurnal changes in levels of activity in the neurotransmitter and neuroendocrine systems which continue to be the focus of much of our clinical investigation. We intend to investigate this hypothesis in relation to manic-depressive mood cycles and in relation to other physiological (temperature, sleep stages), behavioral (activity), and neuroendocrine (prolactin, growth hormone, cortisol, and TSH) rhythms. Since the pineal is regulated by beta-adrenergic receptors, and since melatonin is a product of and a receptor-blocker of serotonin, we will have an output measure of adrenergic

receptor sensitivity and will be determining levels of a possible endogenous regulator of serotonergic receptor sensitivity.

Dr. Lewy's highly sensitive gas chromatographic-mass spectroscopic (GS-MS) assay, which can measure human melatonin in 1 cc of whole blood, will permit characterization of the 24-hour nyctohemeral rhythm at high resolution (every 15 minutes). Normal and pathological rhythms can then be more easily differentiated. The effect of environmental lighting, diet, activity, sleep and psychotropic drugs will also be explored.

The GC-MS assay will also be used to validate the specificity, precision, and accuracy of our radioimmunoassay (RIA). Both assays will facilitate rapid and efficient processing of serum samples, as well as CSF and urine samples. Rhesus monkeys, cannulated in the third ventricle, will provide CSF under various environmental and pharmacological conditions.

8. Our earlier serendipitous observations that antidepressant medications alter the time course of recurrent affective disorders have now been extended to a larger group of patients. Tricyclics, MAOIs and cyproheptadine have markedly accelerated mood cycle frequency from one cycle every few months to one cycle every few weeks or days in seven manic-depressive patients studied longitudinally to date. Therefore, these drugs appear to act by driving rather than counteracting the pathophysiological process underlying recurrent depressive illness. This hypothesis will be explored in outpatient studies designed to assess the long-term effects of tricyclics on the frequency of affective episodes in patients maintained on lithium, in intensive inpatient studies of the effects of tricyclics on neuroendocrine rhythms, and in animal studies of the effects of antidepressants on the spontaneous (free-running) frequency of hypothalamic biological oscillators which drive the activity-rest cycle.

Carmen, J.S., Post, R.M., Buswell, R. and Goodwin, F.K.: Negative Effects of Melatonin on Depression. Am. J. Psychiat. 131:1181-1186, 1976.

Wehr, T.: The Switch Process in Manic-depressive Psychosis. II. Phenomenology: A Dynamic Point of View. Ann. Int. Med., in press.

Wehr, T. and Goodwin, F.K.: Catecholamines and Depression. In Burrows, G. (Ed.): Handbook on Depression. Amsterdam, Exerpta Medica, 1977, pp. 329-348.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00449-03 CP

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Outpatient Followup Studies of Manic-depressive Patients and Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
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	Philip Gold	Chief, Outpatient & Follow-Up Unit	CP/NIMH
	Athanasios Zis	Guest Worker	CP/NIMH
	Elliot Gershon	Chief, Unit on Psychogenetics	BP/NIMH
	Gay Grover	Clinical Nurse Expert	CC Nursing

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Psychobiology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

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[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [X] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient facility was established as a treatment and research resource for a variety of studies in seven key areas which include the following: a) biological investigations of the "well-state"; (b) the usefulness of couples group therapy in the management of recurrent affective illness; (c) assessment of interpersonal relationships and family dynamics in bipolar families; (d) outcome studies; (e) prophylactic efficacy of various drug regimens; (f) the evaluation of genetic markers in affective illness; (g) the phenomenology of multigenerational families with affective illness. The project on couples group therapy have revealed significantly better social functioning, family interaction and mental status in those patients maintained on lithium carbonate in conjunction with couples group therapy compared to manic-depressive patients maintained adequately on lithium, but not receiving adjunctive couples therapy. Long-term outcome has been found to correlate with age of onset, rapidity of cycles and family history.

PHS-6040

A major focus of the Outpatient and Follow-Up Unit this year has been the recruitment of a larger number of patients with major affective illness, resulting in an increase in the clinic population by over 150% over the last 12 months. This enlarged census permits a series of integrated phenomenological, psychological, pharmacological and neurobiological studies. Our primary purpose has been to set up a facility which would be a source of patients for a variety of studies in seven key areas: (a) biological investigations of the "well-state"; (b) a continuing exploration of the usefulness of couples group therapy in the management of recurrent affective illness; (c) assessment of interpersonal relationships and family dynamics in bipolar families; (d) outcome studies; (e) prophylactic efficacy of various drug regimens; (f) the study of multigenerational families with affective illness and (g) the evaluation of genetic markers in affective illness. Patients are admitted to the outpatient department following discharge from the inpatient unit, or directly from the community, and may be involved at one of several levels including a full treatment program (maintenance, medication plus individual or group psychotherapy), medication maintenance, or research followup (care provided in the community).

(A) Biological studies of the "well-state": certain biological findings in depressed patients seem to persist in the immediate recovery period while the patient is still hospitalized. The theoretical implications of these findings have underlined the importance of obtaining data at long-term followup, after full recovery can be presumed. For this purpose, patients are restudied at periods ranging from 6 to 12 months following discharge. Neuroendocrine studies (see project #Z01 MH 0045-02 CP) are of particular interest since they are especially suitable to outpatients. A related project involves the exploration of the neuroendocrine effects of long-term lithium.

(B) Exploration of the usefulness of couples group therapy as an adjunct to the maintenance use of lithium in the prevention of recurrence in affective illness: This project, under the direction of Yolande Davenport, is evaluating outcome in married manic-depressive patients maintained in conjoint couples group therapy compared to manic-depressive patients maintained adequately on lithium but not receiving adjunctive couples therapy. Post-hospital functioning rated on a multi-item scale developed from a previous followup study of manic-depressive illness done by this group showed significant differences between the two groups with a more favorable outcome being associated with the patients receiving both lithium carbonate and couples group therapy. The study is now being extended to include full randomization of patient assignment.

(C) The family dynamics of bipolar patients, an area of comparative research neglect, has been a major focus this past year in the work with the married couples psychotherapy group. Having delineated some of the maladaptive, interpersonal behavioral patterns of the bipolar patient and spouse, the family systems of patients in this group with multigenerational histories of affective disorder are being examined by Yolande Davenport and

Philip Gold to provide further information regarding familial conditions which may contribute to perpetuating affective systems. Further information is obtained from the addition this year of monthly art therapy sessions with the couples. The impact of manic-depressive illness on the adolescent is the focus of a collaborative study in the outpatient clinic by Yolande Davenport, Gabrielle Carlson and Kay Jamison of the Neuropsychiatric Institute, Department of Child Psychiatry, UCLA.

(D) Followup studies of manic-depressive patients: in this group of studies (some done in collaboration with the Unit on Psychogenetics, BPB) the relationships between past history, phenomenology of the acute illness and long-term outcome are examined. To date we have demonstrated relationships between outcome and a variety of illness parameters including age of onset, rapidity of cycles, and family history.

(E) Under the direction of Philip Gold, Athanasios Zis and Thomas Wehr, an evaluation of the long-term effects of lithium alone, tricyclic antidepressants alone, and lithium plus tricyclic antidepressants in unipolar and bipolar affective illness has been undertaken. Preliminary results indicate that long-term tricyclic administration increases the frequency of cycles in bipolar patients.

(F) Study of multigenerational families: This involves an intensive clinical examination of several families with affective illness in more than two generations, done by Mrs. Davenport. She has found a significantly earlier age of onset in each succeeding generation and is attempting to explore whether environmental factors may account for this finding.

(G) In collaboration with Elliot Gershon (Unit on Psychogenetics, BPB), data are obtained from the outpatients and their families relevant to questions concerning genetic markers. Thus, plasma and red cell samples are taken for assay of blood types, antigens, and certain proteins which are known to be genetically linked in an effort to explore the question of possible genetic markers for the vulnerability to affective illness.

The further course of the outpatient followup studies depends to a large extent on the availability of additional resources to clinically manage this valuable research asset. The variety of studies dependent upon this resource are all in their initial stages and are focused on areas which are relatively unexplored in the field of affective illness.

Carlson, G.A., Davenport, Y.B., Jamison, K.: A Comparison of Outcomes in Adolescent and Late Onset Bipolar Manic-depressive Illness. Am. J. Psychiat., in press.

Chodoff, P. and Goodwin, F.K.: Depression: Psychotherapy or Pharmacotherapy J. Am. Acad. Psychoanalysis 4:275-277, 1976.

Davenport, Y.B., Ebert, M.H., Adland, M.L. and Goodwin, F.K.: Couples Group Therapy as an Adjunct to Lithium Maintenance of the Manic Patient. Am. J. Orthopsychiat. 47:495-502, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00452-02 CP

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Neuroendocrine Studies of Primary Affective Disorder

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	Alfred Lewy	Clinical Associate	CP/NIMH
	Rex Cowdry	Staff Psychiatrist	CP/NIMH
	William Potter	Staff Psychiatrist	CP/NIMH
	Athanasios Zis	Guest Worker	CP/NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Hormones produced by the hypothalamic-pituitary axis are released by neurons modulated by neurotransmitter amines hypothesized to be involved in affective illness; thus, the investigation of the various aspects of neuroendocrine function in patients with affective illness and in normal controls has assumed considerable importance. Several aspects of neuroendocrine function currently under investigation in our unit include plasma studies of baseline levels, circadian patterns, variations during different phases of affective illness, and the effects of a variety of challenge stimuli and clinical and experimental drugs (including amine precursors and specific neurotransmitter agonists and antagonists). In addition, pituitary and hypothalamic peptides are being measured in cerebrospinal fluid. Some findings during the past year include: TSH response to TRH was blunted in unipolar patients compared to both normals and bipolar depressed patients. A correlation between the TSH response and CSF amine metabolites was noted. Urinary free cortisol levels were noted to differ in subgroups of patients with affective illness and to correlate with CSF amine metabolites. CSF amine metabolites were noted to differ significantly in women contingent on the status of their hypothalamic-pituitary-ovarian axis.

Project Description: Z01 MH 00452-02 CP

It is now well established from animal data that the various trophic hormones for the hypothalamic pituitary axis are released under the influence of neuronal systems containing the neurotransmitter amines hypothesized to be involved in the pathophysiology of affective illness. In addition, the relationship between affective illness and a wide variety of endocrine dysfunctions has been known for some time. For these reasons it is of considerable interest to evaluate neuroendocrine function in patients with affective illness and in normal controls. Further reasons for the importance of these studies are: (1) drugs with relatively specific effects on brain amine systems are commonly used in our patients, and (2) a variety of other measures of central amine function will be available from these same patients under the same conditions. Thus, an opportunity is available to add important new information concerning the relationship between neuroendocrine function and brain amine systems in man.

The neuroendocrine studies now underway will be described according to strategies employed, and according to the nature of the question being investigated. First, baseline levels of neuroendocrine hormones are determined in patients with primary affective disorder; this includes examination of circadian patterns, and variations during longer cycles, such as the menstrual cycle or during cycles of depression and mania. Second, the effects of various clinical and experimental drugs (including amine precursors) are of interest. Hormones currently under study are growth hormone, prolactin, LH, FSH, cortisol, and TSH. A special method for integrated circadian analyses employs a constant withdrawal pump that eliminates sampling variations secondary to the episodic nature of neurohormone release. A major emphasis this year has been focused on the evaluation of an integrated test of the adenohypophysis, in which a metabolic stress (arginine infusion) and two hypothalamic releasing factors (TRF and LRF) are given over the course of 6 hours in such a way that their individual effects on pituitary hormones (LH, FSH, growth hormone, prolactin, TSH) can be ascertained. Since each of these systems are regulated by amine pathways which may be different but overlap, the integrated evaluation may allow for a rapid and more precise "window" into brain amine systems. For instance, arginine infusion is thought to release growth hormone via serotonergic mechanisms, while both dopamine and serotonin are thought to be involved (as antagonists) in the TSH response to TRF, and the LH response to LRF involves both serotonin (antagonist) and norepinephrine (agonist).

Of special interest to the study of affective illness is the hypothalamic pituitary adrenal axis. Increased cortisol production and abnormal cortisol rhythm in depressed patients are already established; recent evidence suggests a primary role for the serotonergic system in the regulation of cortisol production. The evaluation of this system involves the administration of a dexamethasone suppression test followed by measurement of urinary free cortisol.

The results obtained from neuroendocrine strategies are being correlated with CSF and urinary amine metabolite data.

A separate neuroendocrine strategy involves evaluating the effects of physiologic doses of various hormonal agents (T_3, testosterone and estrogen) as potentiators of the psychoactive effects of antidepressants.

We are also conducting a comprehensive study of the hypothalamic peptide, vasopressin, in patients with affective illness. Our interest in vasopressin in patients with primary affective disorder is referable to several factors: (1) cognitive abnormalities have been identified in patients with affective illness, regardless of clinical state; vasopressin has been shown in experimental animals to affect specific aspects of learning and memory; (2) electrolyte disturbances have been widely reported in patients with affective illness; vasopressin is an important physiologic determinant of osmotic and electrolyte balance in man; (3) the aminergic neurotransmitters thought to play an important role in affective illness partly regulate the synthesis of vasopressin. In addition, lithium has been shown to block peripheral vasopressin receptors in the kidney, and may well exert part of its action by blocking vasopressin receptors in the central nervous system. Vasopressin is also the precursor of a tripeptide thought to have dopaminergic agonist properties in man and in experimental animals. We are studying vasopressin dynamics, utilizing (1) saline infusion to assess the vasopressin response to increase in osmolality; (2) fructose infusion to determine whether any degree of inappropriate ADH secretion exists in patients after their osmolality has been lowered below the threshold for vasopressin release; (3) the effect of lithium and other agents, particularly specific monoaminergic agonists and antagonists, on functional tests of vasopressin secretion and vasopressin levels; (4) the effects of i.v. dopamine infusion on vasopressin levels; (5) the effects of specific acetylcholine agonists and antagonists on vasopressin levels; (6) the effect of stress on vasopressin, since there is some controversy concerning this particular point.

Vasopressin is also present in considerable concentration in the cerebrospinal fluid, and there is evidence that vasopressin secreted into the ventricular system is synthesized and released from a different locus than vasopressin stored and released by the posterior pituitary. Accordingly, we are measuring vasopressin in the spinal fluid in all of our patients longitudinally during various clinical states and on a variety of psychoactive medications (in collaboration with Gary Robertson of the University of Indiana School of Medicine).

In addition to measuring vasopressin in cerebrospinal fluid, we are embarking on a major project to measure other hypothalamic peptides in the cerebrospinal fluid. We now measure somatostatin, substance P, endorphin, TRH and LH-RH, of which the last three may be neurotransmitters which possess psychoactive properties. Although we feel that the levels of all of these may be important to our understanding of affective illness, we are particularly interested in the level of somatostatin, since we have shown a blunted TSH response to TRH in a particular subgroup of patients which could be accounted for on the basis of increased somatostatin levels. This study is

done in collaboration with Dr. Seymour Reichlin, Tufts University School of Medicine.

We have also begun a study in which we will assess the neuroendocrine effects of a dopamine infusion in patients with affective illness, thus evaluating the sensitivity of the dopamine receptor in the pituitary median emminence. Data from our prior neuroendocrine work suggest that dopamine may have an important role in bipolar affective illness, such that dopaminergic functional activity is decreased in the depressed as compared to the manic phase. Dr. Samuel Yen, University of California School of Medicine, La Jolla, is collaborating in these studies.

In addition to our LH-RH stimulation test, which functionally evaluates the hypothalamic-pituitary-gonadal axis, we are carefully assessing androgen status in our patients by measuring both gonadal and adrenal androgens longitudinally. Clinical observations suggest possible androgen excess during mania; manic patients often develop acneform lesions and show marked hypersexuality, both of which could be attributed to increased androgen status.

In collaboration with Glen Davis of the BPB, we are measuring the effects of narcotic antagonists on neuroendocrine parameters in normal volunteers and patients with affective illness. Recent evidence suggests that opiate agonists raise prolactin, while antagonists lower prolactin; moreover, morphine apparently possesses anti-gonadal function in man.

Recent evidence also suggests that this same cell line, which secretes ACTH, secretes an important endorphin, and that endorphin levels are regulated by many of the same influences which regulate ACTH secretion. Accordingly, we are conducting stimulation tests which have been shown to release ACTH, such as insulin-induced hypoglycemia, to assess their effect on endorphin secretion. Such tests will further elucidate the connection between the hypothalamic-pituitary-adrenal axis and endorphin regulation in man, and perhaps also elucidate a possible functional connection between the endorphinergic system and the monoaminergic system. Dr. Yen is also collaborating in this effort.

In collaboration with Chris Gillin, we are currently studying the effects of the hypothalamic peptides TRH and LH-RH on REM sleep. In addition, we will assess the neuroendocrine effects of these peptides during sleep, comparing responses obtained from TRH and LH-RH infusions on awake subjects during daylight hours.

We have found that growth hormone response to L-DOPA in unipolar depressed patients is blunted when compared to the response in bipolar depressed patients in the baseline state. TSH response to TRH was also blunted in unipolar patients compared to both normals and bipolar depressed patients.

In studies correlating neuroendocrine and other variables, a significant negative correlation was found between the TSH response to TRH and the probenecid-induced accumulation of 5HIAA in the CSF of unipolar depressed patients.

Tests of the hypothalamic-pituitary-adrenal axis have focused on urinary free cortisol (UFC) with and without dexamethasone suppression. Unipolar patients with psychosis have been found to have very high UFC compared to unipolar patients without psychosis, or bipolar depressed patients. Among unipolar patients URC shows a significant positive correlation with CSF 5HIAA after probenecid. A subgroup of patients fail to suppress their UFC after dexamethasone administration. These patients will be placed on cyproheptadine, a serotonin receptor blocker which has been shown to lower serum cortisol and to correct the sleep disorder of Cushing's disease. Although L-tryptophan alone did not alter urinary cortisol, the combination of lithium plus L-tryptophan was associated with a marked rise in urinary free cortisol in the same patients, a finding consistent with animal data, suggesting a synergistic effect of lithium and tryptophan on serotonin synthesis. Chronic lithium administration reduced the urinary free cortisol production, consistent with our CSF data indicating a decrease in serotonin turnover under these conditions.

Gold, P.W. and Goodwin, F.K.: Growth Hormone and Prolactin Response to Levodopa in Affective Illness. Lancet i:965, 1977.

Gold, P.W., Goodwin, F.K., Wehr, T. and Rebar, R.: Thyrotropin Response to Thyrotropin Releasing Hormone in Affective Illness: Correlation with CSF Amine Metabolites. Am. J. Psychiat., in press.

Gold, P.W., Goodwin, F.K., Wehr, T., Rebar, R. and Sack, R.: Growth Hormone and Prolactin Response to Levodopa in Affective Illness. Lancet ii:1307-1308, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01831-01 CP

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Structural and Functional Relationships of a Neuronal and Glial Form of Enolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Frederick Goodwin	Chief	CP/NIMH
	Eberhard Trams	Head, Section on Metab. & Physiol.	LNC/NIMH
	Marshal Nirenberg	Chief	IR BG/NHLBI
	Gilbert Omenn		U. of Wash.
	Athanasios Zis	Guest Worker	CP/NIMH
	Irwin Kopin	Chief	LCS/NIMH
	Donald Schmechel	Medical Officer	LCS/NIMH

COOPERATING UNITS (if any)
Laboratory of Neurochemistry and Laboratory of Clinical Science, NIMH;
Laboratory of Biochemical Genetics, NHLBI

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, MD. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This study involves the characterizations of two proteins we have isolated from brain. The proteins are neuron specific enolase (NSE) and non-neuronal enolase (NNE). Both proteins are isoenzymes of the glycolytic enzyme enolase in brain. NSE is localized in neurons and NNE in glia. The functional characterization and comparison is expected to provide information relating to the nature and regulation of brain energy metabolism at the neuronal and glial level. Both proteins have been purified; they are distinct kinetically and probably functionally. The neuronal enzyme is stable in the presence of salts such as KCL and NaCl, whereas the glial enzyme is inactivated. Studies of NSE levels in various neuroblastoma cells have indicated that this protein is a molecular correlate of differentiation and that its levels are responsive to dibutryl cAMP treatment.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MH 01831-01 CP

A viable approach to the study of brain function at the molecular level is the structural and functional characterization of neuron specific proteins.

A soluble acidic protein has been isolated in pure form from rat, cat and human brain. This protein has been structurally characterized with the results indicating it is a dimer of two 40,000 M.W. subunits. A specific immunoassay has been developed for the protein which made it possible for us to demonstrate that it is specific to nervous tissue. Immunocytochemical analysis has further revealed that the protein is only present in neurons, and accordingly, it has been designated neuron specific protein or NSP.

An intensive effort directed at elucidating the function of NSP in nervous tissue has revealed that it is a neuron specific form of the glycolytic enzyme enolase. In accordance with this discovery, the protein is now designated neuron specific enolase or NSE.

Brain tissue, particularly the neuron, is among the most metabolically active tissue in the body. More than 20% of total tissue oxygen consumption is accounted for by the brain although it represents only about 2% of the total body weight. Glycolysis is a very important pathway in brain metabolism since the only substrate of energy metabolism able to traverse the blood-brain barrier is glucose. Any alteration of glycolytic flux would therefore effect oxidative metabolism (TCA cycle) as well as causing a drastic alteration in the entire energy generating (ATP generating) process. The regulation of glycolysis in the neuron therefore becomes critical since an enormous amount of ATP is required for such diverse neuronal life processes as maintenance of membrane polarity, transmitter synthesis, release, reuptake, etc.

With the discovery that NSE is a neuron specific form of enolase it became important to determine if other forms of enolases exist in brain. Since NSP is strictly neuronal it follows that glial cells should have another form of the enzyme. We have determined that three forms of enolase exist in rat brain, one of which is NSE. Very recently we have purified a second enzyme species and raised specific antibodies against it. Immunocytochemical studies utilizing the peroxidase-anti-peroxidase staining technique have recently shown that this other enolase form is only found in glial cells. It is designated non-neuronal enolase or NNE.

NSE and NNE have been structurally analyzed with the results indicating that the two proteins are composed of different subunits. NSE is a dimer designated $\gamma\gamma$ and NNE is a dimer designated $\alpha\alpha$. We have therefore established that the two proteins are the product of separate genes. The third brain enolase isoenzyme has been shown to be a hybrid of NSE and NNE ($\alpha\gamma$). With the structural comparison of the brain enolase isoenzymes completed, we are now in the process of functionally characterizing the neuronal and glial enzymes. A comparison of these two enzyme forms is expected to provide information relating to the nature and regulation of energy metabolism in

brain. Data generated to date indicate that NSE and NNE are distinct kinetically with NSE displaying a three-fold greater affinity for Mg (a cofactor of the enolase reaction) than NNE. Stability studies have also indicated that NNE is dissociated and inactivated in the presence of salts such as KCL, KBr and NaCl with NSE being highly resistant towards such treatments. The picture emerging is that NSE is a more efficient enzyme kinetically than NNE. The neuronal enolase appears well suited to the neuron since the relatively pronounced ionic fluxes occurring in this cell type would not effect NSE as they would the glial enzyme. Further functional studies are in progress directed towards elucidating factors responsible for controlling the synthesis of NSE and NNE. In these studies each protein is dissociated into its component subunits, a process leading to total loss of all activity. This inactivated material is then renatured with a regain of enzyme activity. Factors affecting the renaturating process are being studied using both pure NSE and NNE. This in vitro dissociation-reassociation system can be taken as a model for in vivo post-translational regulatory mechanisms. A comparison of the factors required for the reassociation and reactivation of NSE and NNE should yield interesting insights into the control of energy metabolism in each respective cell type.

During the last year studies have been done involving the characterization of brain enolases in neuroblastoma cells and its relationship to neuronal differentiation. In collaboration with Dr. Eberhard Trams and Carl Lauter of NINCDS, NSE has been shown to be very low in 3 lines of neuroblastoma cells with NNE being very high. NSE is apparently a marker for differentiated neurons since treatment of the cells with Dibutryl cAMP increases NSE levels markedly. The tissue culture experiments are being extended using immunocytochemical staining procedures in an effort to try and determine if only certain neurons in culture are producing NSE or if all cells are making very low levels of the protein. The methodology involved in immunologically staining neuroblastoma cells has now been worked out with preliminary results indicating a differential staining pattern suggesting that some cells are making more NSE than others. An effort will be made to correlate the degree of morphological differentiation with the level of NSE. Studies are also planned in which cultures of non-cancerous peripheral nervous tissue will be utilized. NSP and NNE levels will be measured under different physiological conditions (stimulation, NGF treatment, etc.) in an effort to address the question of how these two proteins are regulated.

We have very recently determined that NSE is present in the adrenal medulla with none present in the cortex. Studies using the radioimmunoassay are presently in progress in an effort to determine if various hormone and stress paradigms can alter the levels of NSE. The adrenal medulla is the only tissue other than the neuron where NSE has been found. The cells of which the adrenal medulla is composed have been thought to be neuron-like and the presence of NSE in these cells confirms this postulation. The adrenal studies are being done in collaboration with Dr. Irwin Kopin and Donald Schmechel.

Marangos, P.J., Goodwin, F.K., Parma, A., Lauter, C. and Trams, E.: Neuron Specific Protein (NSP) in Neuroblastoma Cells: Relation to Differentiation. Brain Res., in press.

Marangos, P.J. and Zomzely-Neurath, C.: Determination and Characterization of Neuron Specific Protein (NSP) Associated Enolase Activity. Biochem. Biophys. Res. Commun. 68:1309, 1976.

Marangos, P.J., Zomzely-Neurath, C. and Goodwin, F.K.: Structural and Immunological Properties of Neuron Specific Protein (NSP) from Rat, Cat and Human Brain: Comparison to Bovine 14-3-2. J. Neurochem. 28:1097, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01832-01 CP

PERIOD COVERED

July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of Energy Metabolism in Brain: The Glycolytic Enzyme

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Frederick Goodwin	Chief	CP/NIMH
	Donald Schmechel	Medical Officer	LCS/NIMH
	Milton Brightman	Head, Section on Neurocytology	LNNS/NINCDS
	Irwin Kopin	Chief	LCS/NIMH
	Spiros Constantinides		U. of R.I.
	Louis Sokoloff	Chief	LCM/NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Psychobiology Branch

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NIMH, ADAMHA, NIH, Bethesda, MD. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

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☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The discovery in our laboratory of a neuron specific form of the glycolytic enzyme enolase (NSE) which is structurally and functionally distinct from the non-neuronal enolase (NNE) localized in glial cells (see project #Z01 MH 01831-01 CP) has prompted us to further explore the enzymes of this crucial metabolic pathway in brain tissue. The glycolytic enzymes aldolase and hexokinase have also been implicated as having brain specific isoenzymes. Work is underway to isolate these two enzymes from brain tissue and to determine if brain specific forms exist. Antisera to the purified proteins will be used immunohistochemically to stain brain tissue to determine if there are neuronal and glial specific forms of other glycolytic enzymes. The work concerning brain specific glycolytic enzymes will be integrated with investigations currently in progress at NIH concerning the non-metabolizable glycolytic substrate 2-deoxyglucose. The two methodologies will corroborate and compliment each other in understanding brain energy metabolism at the cellular level.

Project Description: Z01 MH 01832-01 CP

Insufficient attention has been given to the importance of brain energy metabolism and its obvious unique characteristics. A summary of the unique metabolic characteristics of brain include the facts that it is the most metabolically active organ in the body, its only energy substrate is glucose and it is extremely sensitive to lack of oxygen or glucose with short periods of deprivation leading to coma and death.

Subtle changes in the energy flux of the brain or localized areas therein can be expected to cause dramatic behavioral changes. That such changes occur and are a component of the pathology in various mental disorders has been suggested previously. The absolute dependence of brain on glucose as an energy source confers a critical importance to glycolysis in this tissue since this pathway becomes a necessary processing step for oxidation metabolism and ATP generation. Glycolysis in brain can for the above reasons be thought of as a gating mechanism controlling energy production. The enzymes involved in this pathway and their regulation might therefore be predicted to be unique. This has already been demonstrated with one enzyme of glycolysis (enolase) which has been demonstrated to exist as a neuron specific isoenzyme having distinct structural and functional properties.

The work done in our laboratory concerning the elucidation of a neuron specific form of the glycolytic enzyme enolase (NSE) has prompted us to study brain glycolytic enzymes more closely. A brain specific form of the key glycolytic enzyme aldolase has been shown to exist in rat and rabbit. The brain contains five forms of aldolase. Two subunits exist, A and C, with the five possible combinations of a pentamer being A^4, A^3C, A^2C^2, AC^3 and C^4. The C^4 protein is only found in brain, indicating that the C polypeptide is only made in nervous tissue, a situation remarkably similar to NSE.

In collaboration with Dr. Irwin Kopin and Donald Schmechel, we have begun work on isolating the C^4 aldolase from rat brain. The enzyme is being characterized so that a specific antiserum can be raised against it. The relevant immunohistochemical experiments will be performed with the anti-brain aldolase serum in an effort to determine the cellular localization of C^4 aldolase in brain tissue. The A^4 aldolase will be treated in a similar manner. These experiments should reveal whether brain aldolase is strictly localized in specific cell populations. If a neuronal and glial form of aldolase exists then it will become apparent that these two cell types have specific variations of the glycolytic pathway. The more neuron specific enzymes of glycolysis that can be isolated and characterized, the better the prospects for understanding how energy metabolism is regulated in this cell.

Similar studies are underway with the glycolytic enzyme hexokinase, which has been reported to have a component in brain that is not found in other tissues. The aid of other investigators has been enlisted in searching for brain specific isoenzymes. Dr. S. Constantinides at the University of Rhode Island has agreed to collaborate in the project by exploring brain

tissue for a specific isoenzyme of glyceraldehyde-3-phosphate dehydroxynase (GAPD), a key glycolytic enzyme. If a specific form exists it will be treated in the same way as the other enzymes with its cell localization determined. It is hoped that eventually a pattern will emerge which will reveal whether glycolysis is composed of cell specific isoenzymes in the neuron.

Attempts are being made to coordinate apply the ongoing work concerning brain glycolytic enzymes with the work being conducted in other laboratories at NIH concerning the non-metabolizable glycolytic substrate 2-deoxyglucose. Autoradiographic studies utilizing this compound can illustrate areas of high metabolic activity in brain. Immunocytochemical analysis using antibodies to the glycolytic enzymes NSE and NNE and other neuronal or glial enzyme forms will compliment and extend the 2-deoxyglucose technology in that specific cell types can be examined. The 2-deoxyglucose will be taken up by all cells, neurons and glia, whereas the immunocytochemical method will enable us to look at each of these cell types individually.

Marangos, P.J. and Zomzely-Neurath, C.: Determination and Characterization of Neuron Specific Protein (NSP) Associated Enolase Activity. Biochem. Biophys. Res. Commun. 68:1309, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01833-01 CP

PERIOD COVERED

July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Brain Specific Antigens and Neurotransmitter Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Donald Schmechel	Medical Officer	LCS/NIMH
	Milton Brightman	Head, Section on Neurocytology	LNNS/NINCDS
	Irwin Kopin	Chief	LCS/NIMH
	Michael Brownstein	Medical Officer (Research)	LCS/NIMH
	Athanasios Zis	Guest Worker	CP/NIMH
	Christian Anfinsen	Chief	LCB/NIAMDD
	Marius Valsamis	Neuropathologist	N.Y. Med. Coll.
	Alfred Lewy	Clinical Associate	CP/NIMH
	Irwin Chaiken	Research Chemist	LCB/NIAMDD

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Section on Neurocytology, LNNS/NINCDS; Laboratory of Chemical Biology, LCB/NIAMDD.

LAB/BRANCH

Clinical Psychobiology Branch

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Recent work in our laboratory has established that brain enolase has a neuronal isoenzyme and a glial form and that these two proteins are specific biochemical markers for the respective cell types. These two proteins are used as an index of neuronal and glial cell densities and in studies involving the differentiation of various neuronal populations. The studies utilize the peroxidase anti-peroxidase staining methodology. Antibodies have also been raised to melatonin and its localization is being determined in brain and peripheral tissues. Antibodies specific to PNMT and glutamic acid decarboxylase (GAD) are also being prepared in an effort to map their location in brain. Neurotransmitter receptor studies are also in the developing stages where dopaminergic and enkephalinergic receptors will be analyzed as to their binding properties in response to various neuroleptic drugs. This study involves the development of specific receptor binding assays, and attempts at isolating these receptor molecules in pure form.

Project Description: Z01 MH 01833-01 CP

Work in our laboratory has established that the glycolytic enzyme enolase has a neuron specific (NSE) and glial specific (NNE) isoenzyme which represent specific molecular markers for the respective cell types. The peroxidase-anti-peroxidase immunohistochemical technique utilizing the antiserum to NSE and NNE has been employed to establish this. Studies are presently in progress attempting to quantify each protein in the various neuronal and glial subclasses. Developmental studies are also in progress in which the time course of NSE and NNE appearance is being studied in embryonic and post-gestation rat and human brain tissue. Radioimmunoassay is being employed as a corroborative technique in these studies.

Behavioral and pharmacological studies are also underway where various activity, training and drug challenges are tested for effects on both NSE and NNE levels. The protein levels are quantitated immunohistologically and by radioimmunoassay.

In an effort to develop an immunohistochemical program at NIMH, in collaboration with Dr. Donald Schmechel, we are acquiring a number of antibodies to brain antigen in order to map their location in brain. The work already in progress establishing that NSE and NNE are specific biochemical markers for neuronal and glial cells respectively has spearheaded this effort. In this regard we have prepared an antibody to melatonin and developed a specific radioimmunoassay. This antibody will be used for studies aimed at localizing this proposed neurohormone. The radioimmunoassay will allow measurement of melatonin using the stereotaxic brain punch technique. Collaborations have also been established with groups preparing antibodies to PNMT and GAD. A knowledge of localization of these brain antigens will increase our understanding of their functions in nervous tissue.

Some clinically related projects concerning brain specific antigens are already in progress. Animal studies are being planned in which the central dopaminergic and adrenergic receptors of rat brain will be characterized with respect to drug administration. Possible changes in binding affinities and actual receptor number will be investigated. Autopsied human brain tissue from schizophrenic patients has been obtained and will undergo studies of receptor characterization.

As part of a long range receptor program planned in our laboratory the purification of membrane receptors will be undertaken. Initially using methodologies similar to those utilized for the purification of the cholinergic receptor the dopaminergic and adrenergic receptors will be processed. This will be a difficult process due to the insoluble matrix in which the receptor is imbedded. It is also likely that upon removal of the receptor molecule from the membrane, its binding properties will be lost. We feel this is an important undertaking since isolation of a neurotransmitter receptor will make possible its detailed characterization such as the mechanism and regulation of ligand binding. It is also possible that during receptor isolation the postulated presynaptic receptor species will be detected or

that multiple receptor populations will emerge. The preparation of antibodies against purified receptors would be a major breakthrough in that it would make possible direct visualization of their location as well as provide a specific blocking agent for neurophysiological studies.

Clinical studies involving the brain enolases are in progress. These studies bear on the autoimmune hypotheses of various brain disorders (such as schizophrenia, Altzheimer's disease and various demyelinating diseases) and are being done in collaboration with Dr. Marius Valsamis and Dr. Athanasios P. Zis. The autoimmune nature of diseases such as myasthenia gravis have already been documented; it appears that antibodies to the nicotinic cholinergic receptor are central to the pathophysiology. Sera from patients is being tested for its ability to bind labeled NSE and NNE. The availability of these specific neuronal and glial antigens affords the unique capability of assessing whether any of these illnesses involves a specific immunochemical lesion. Autopsied brain tissue from senile dementia patients has also been obtained for measurement of NSE and NNE tissue levels. If antibodies to either protein are detected in any disorder, their assay would provide a quick and sensitive diagnostic tool and possibly detect predispositions towards such disorders before gross symptoms appear. This theoretical framework will be applied generally using antisera to as many brain antigens as possible in an effort to explore the possible immunodiagnostic value of specific antigens and antisera. The volume of speculative, suggestive proposals concerning immunodiagnostics and immunotherapy far exceeds the amount of scientific fact. This is largely due to the lack of specific antigens and antisera available for clinical hypothesis testing. The antigens and antisera for NSE and NNE are now available for such screening purposes and efforts are being made towards obtaining additional material. Immunopathological studies of brain disorders are expected to yield considerable information about the molecular basis of brain function and dysfunction.

Publications: ZO1 MH 01833-01 CP

Pickel, V.M., Reis, D.J., Marangos, P.J. and Zomzely-Neurath, C.: Immunocytochemical Localization of Nervous System Protein (NSP-R) in Rat Brain. Brain Res. 105:184, 1976.

St-Laurent, J., Roizen, M.F., Beckmann, H., Miliaressis, E., Goodwin, F.K. and Jacobowitz, D.M.: Neurochemical Changes in Discrete Areas of the Rat Brain After Self-stimulation from the Area Ventralis Tegmenti. In Wanquier, A. and Rolls, E.T. (Eds.): Brain-Stimulation Reward. The Netherlands, North Holland Publishing Company, 1976, pp. 283-289.

Zomzely-Neurath, C., Marangos, P.J., York, C. and Heimowitz, N.: The Effect of a Behavioral Task on Neuronal and Glial Specific Proteins. Neurochem. Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00421-15 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Neuroanatomy and Metabolism of Biogenic Amines in the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. Settler	SKF Labs
	R. Kvetnansky, Visiting Scientist	LCS NIMH
	R. McCarty, Research Associate	LCS NIMH
	J. Ribas	AFRRI
	D. Carpenter, Chief, Dept. Neurobiology	AFRRI

COOPERATING UNITS (if any)
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Department of Neurobiology, Armed forces Radiobiology Research Institute, Bethesda, Maryland

LAB/BRANCH
Laboratory of Clinical Science

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Section on Pharmacology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to develop micromethods for the measurement of normally occurring biogenic amines, receptors and enzymes; to localize these amines and receptors and enzymes in peripheral tissues and specific brain nuclei and determine the effect of hypertension, endocrine manipulation and psychotropic drugs.

Phenylethanolamine was found to be present in the nervous system of Aplysia. Selective increases in GAD (glutamic acid decarboxylase) occurred in the ipsilateral caudate and substantia nigra after chemical lesion of A_9 and A_{10}, indicating a relationship between the GABAergic and dopaminergic pathways. The dopamine-sensitive adenylate cyclase in the substantia nigra is localized outside the dopaminergic cells.

Inhibition of catecholamine synthesis results in changes of serotonin metabolism in selective raphe nuclei.

The activity of the adrenaline-forming enzyme, PNMT, is relatively high in the septal area.

Project Description:

Objectives: To study the localization and metabolism of biogenic amines in discrete areas of the brain and determine the effect of anatomically specific lesions, endocrine manipulations and psychotropic drugs.

Methods Employed: Enzymatic, radiometric, microanatomical and pharmacological.

Major Findings: Phenylethanolamine was found to be present in the nervous system of Aplysia. Iontophoretic application of phenylethanolamine to single Aplysia cells resulted in specific electrical responses and changes in membrane permeability, indicating the possibility of specific phenylethanolamine receptors in those cells.

The dopamine projections from the areas A_9 and A_{10} to the forebrain have been quantitatively studied. A relative specificity for the 6-hydroxydopamine lesion was demonstrated. Selective increases in GAD (glutamic acid decarboxylase) occurred in the ipsilateral caudate and substantia nigra, indicating a relationship between the gabaergic and dopaminergic pathways.

The dopamine-sensitive adenylate cyclase in the substantia nigra is localized outside the dopaminergic cells.

Catecholamines have been shown to be present in the raphe nuclei of the rat. Inhibition of catecholamine synthesis results in changes of serotonin metabolism in selective raphe nuclei, indicating the possibility of an interaction between the two systems in specific brain areas.

Concentrations of catechol-O-methyltransferase, histamine N-methyltransferase and monoamine oxidase activities have been measured in more than 50 areas of the rat brain.

Histamine levels have been found to be high in septal areas and in some raphe nuclei. Serotonin has been shown to be present in catecholaminergic areas of the brain stem, like the areas A_1 and A_2, related to the regulation of the blood pressure.

The activity of the adrenaline-forming enzyme, PNMT, is relatively high in the septal area.

Acute immobilization stress produces a decrease in NE and E content in areas of the anterior hypothalamus physiologically connected with the function of the adrenal medulla.

Significance to Biomedical Research: A relationship between gabaergic and dopaminergic pathways appears to occur in localized areas of the nigro-striatal pathway, supporting a physiological interaction of these two systems in specific brain areas.

In the substantia nigra, the dopamine-sensitive adenylate cyclase has been shown to occur outside the dopaminergic cells, indicating the possibility of the existence of post-synaptic dopaminergic receptors in this region. The substantia nigra could be considered as another site of action for neuroleptic and anti-Parkinson drugs.

The formation of catecholamines in some of the raphe nuclei, and the changes in serotonin metabolism after blockade of the synthesis of noradrenaline indicate that catecholamines can regulate the metabolism of serotonin in the brain.

Changes in catecholamine levels after acute stress are localized to specific areas of the hypothalamus. The possibility exists of a role for hypothalamic catecholamines in the control of the adrenal function.

The minor biogenic amine, phenylethanolamine, could play a role as a neurotransmitter in the nervous system of invertebrates.

Proposed Course of Project: The influence of changes in catecholamine metabolism on the turnover and formation of serotonin in specific brain areas will be examined. The effect of acute and chronic stress on the metabolism of brain catecholamines will be further studied, with special emphasis on the anterior hypothalamic and brain stem areas related with adrenal function. A detailed mapping of histamine in specific brain areas will be performed. The influence of endocrine manipulations and stress on serotonin and catecholamine metabolism will be further studied.

Publications:

Saavedra, J.M., Grobecker, H., and Axelrod, J.: Biochemical and morphologic study of catecholamine metabolism in spontaneously hypertensive rats. Mayo Clinic Proc. 52: 391-394, 1977.

Saavedra, J.M.: Localization of Biogenic-Amine-Synthesizing Enzymes in Discrete Hypothalamic Nuclei. In Stumpf, W.E. and Grant, L.D. (eds): Anatomical Neuroendocrinology. Basel, S. Karger, 1975, pp. 397-400.

Saavedra, J.M.: The Role of Methylating Enzymes in Brain Function. In Youdim, M. and Lovenberg, W. (eds): Essays in Neurochemistry and Neuropharmacology, Volume 1, Chapter 1. London, John Wiley & Sons, 1976, pp. 2-41.

Saavedra, J.M., Grobecker, H., and Zivin, J.: Catecholamines in the raphe nuclei of the rat. Brain Res. 114: 339-345, 1976.

Saavedra, J.M., Brownstein, M.J., Kizer, J.S., and Palkovits, M.: Biogenic amines and related enzymes in the circumventricular organs of the rat. Brain Res. 107: 412-417, 1976.

Saavedra, J.M., Brownstein, M.J., and Palkovits, M.: Distribution of catechol-O-methyltransferase, histamine N-methyltransferase and monoamine oxidase in specific areas of the rat brain. Brain Res. 118: 152-156, 1976.

Kebabian, J.W., and Saavedra, J.M.: Dopamine-sensitive adenylate cyclase occurs in a region of substantia nigra containing dopaminergic dendrites. Science 193: 683-685, 1976.

Saavedra, J.M., Ribas, J., Swann, J., and Carpenter, D.O.: Phenylethanolamine: A New Putative Neurotransmitter in Aplysia. Science 195: 1004-1006, 1977.

Saavedra, J.M., and Liuzzi, A.: Nerve Growth Factor: Effects on 5-hydroxytryptamine and Phenylethanolamine N-methyl Transferase in the Superior Cervical Ganglion of the Rat. In Eranko, O. (ed.): SIF Cells, Fogarty International Center Proceedings No. 30. Washington, D.C., U.S. Government Printing Office, 1976, Chapter 10, pp. 124-130.

Saavedra, J.M.: Distribution of Serotonin and Synthesizing Enzymes in Discrete Areas of the Brain. Fed. Proc. 36: 2134-2141, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00422-06 LCS

PERIOD COVERED: July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical and Pharmacological Studies on the Pineal Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Zatz, Senior Staff Fellow	LCS NIMH
	R. F O'Dea, Guest Worker	LCS NIMH
Other:	C. Gágnon, Guest Worker	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Clinical Science

SECTION: Section on Pharmacology

INSTITUTE AND LOCATION: NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the pineal as a model to learn how nerves interact with responsive cells.

There is a prominent circadian rhythm in the postjunctional sensitivity of the pineal to stimulation by β-adrenergic agonists. This variation is regulated at multiple sites, each of which is involved in the generation of the pineal's response to stimulation via cyclic AMP and N-acetyltransferase.

Both cyclic nucleotides are released from the pineal: cyclic AMP is released by parenchymal cells whereas cyclic GMP is released from the noradrenergic nerve endings in the gland. The release of cyclic GMP was also demonstrated from the peptidergic nerve endings in the posterior pituitary.

Project Description:

Objectives: To use the pineal as a model to learn how nerves interact with responsive cells.

Methods Employed: Biochemical, pharmacological, and radioactive tracer techniques.

Major Findings: There is a prominent circadian rhythm in the postjunctional sensitivity of the pineal to stimulation by β-adrenergic agonists. This variation is regulated at multiple sites, each of which is involved in the generation of the pineal's response to stimulation via cyclic AMP, and N-acetyltransferase. Work with classical adrenergic agonists as well as choleragen, which directly stimulates adenylate cyclase, demonstrated alterations in the β-adrenergic binding sites, adenylate cyclase, and cyclic AMP-dependent protein kinase, both in response to stimulation and in the regulation of sensitivity.

Unlike cyclic AMP, which is involved in postsynaptic events, cyclic GMP was shown to be involved in presynaptic events. Both cyclic nucleotides are released from the pineal; cyclic AMP is released by parenchymal cells whereas cyclic GMP is released from the noradrenergic nerve endings in the gland. The release of cyclic GMP was also demonstrated from the peptidergic nerve endings in the posterior pituitary. The release of cyclic GMP from nerve endings is stimulated by noradrenaline, requires Ca^{++} and is related to the oxocytotic release of neurotransmitters.

Significance to Biomedical Research: Elucidation of the circadian rhythms in sensitivity of the pineal gland provides insight into the regulation of the responsiveness of excitable cells. The role of cyclic nucleotides in presynaptic function should provide a better understanding of the regulation of neurotransmitter release.

Proposed Course of Project: The role of cyclic nucleotides in the regulation of neurotransmitter release, and in the responses of cells of neurotransmitters, will be explored further.

Publications:

Zatz, M., Kebabian, J.W., Romero, J.A., Lefkowitz, R.J., and Axelrod, J.: Pineal β-adrenergic Receptor: Correlation of Binding of ^{3}H-(1)-alprenolol with Stimulation of Adenylate Cyclase. J. Pharmacol. Exp. Ther. 196: 714-722, 1976.

Zatz, M., Romero, J.A., and Axelrod, J.: Diurnal Variation in Requirement for RNA Synthesis in the Induction of Pineal N-acetyltransferase. Biochem. Pharmacol. 25: 903-906, 1976.

O'Dea, R.F., and Zatz, M.: Catecholamine-Stimulated Cyclic GMP Accumulation in the Rat Pineal: Apparent Presynaptic Site of Action. Proc. Nat. Acad. Sci. USA 73: 3398-3402, 1976.

Zatz, M., and O'Dea, R.F.: Regulation of Protein Kinase in Rat Pineal: Increased V_{max} in Supersensitive Glands. J. Cyclic Nucleo. Res. 2: 427-439, 1976.

Zatz, M., and O'Dea, R.F.: Efflux of Cyclic Nucleotides from Rat Pineal: Release of Cyclic GMP from Sympathetic Nerve Endings. Science 197 174-175, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 00423-04 LCS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Protein Carboxymethylation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. O'Dea, Guest Worker	LCS NIMH
	C. Gagnon, Guest Worker	LCS NIMH
	O. H. Viveros, Visiting Scientist	LCS NIMH
Other:	E. Schiffman	LBDA NIDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the properties, distribution and physiological role of protein carboxymethyl transferase and its acceptor proteins.

Protein carboxymethylase has been found mainly in the cytosol of the three pituitary lobes (posterior, intermediate and anterior). The acceptor proteins are highly localized in the intravesicular fractions. Treating rats with high salt diets results in an increase of protein carboxymethylase and a marked decrease of acceptor proteins in the posterior pituitary. Protein carboxymethylase and its acceptor protein has been found in red cells, platelets and leukocytes. Thrombin stimulates protein carboxymethylation in platelets. Chemotaxic bacterial peptides cause a rapid, transient increase in protein carboxymethylation in leukocytes. There appears to be a direct correlation between the ability of the bacterial peptides to attract the leukocytes and protein carboxymethylation.

Project Description:

Objectives: To study the properties, distribution, and physiological role of protein carboxymethyl transferase and its acceptor proteins.

Methods Employed: Enzymatic, radiometric and endocrinological.

Major Findings: Protein carboxymethylase has been found mainly in the cytosol of the three pituitary lobes (posterior, intermediate and anterior). Except for the anterior lobe the enzyme is absent in vesicles. The acceptor proteins are highly localized in the intravesicular fractions. Treating rats with high salt diets results in an increase of protein carboxymethylase and a marked decrease of acceptor proteins in the posterior pituitary. The methylation of neurophysin is increased when it binds to oxytocin. Protein carboxymethylase and its acceptor protein has been found in red cells, platelets and leukocytes. Thrombin stimulates protein carboxymethylation in platelets. Chemotaxic bacterial peptides cause a rapid transient increase in protein carboxymethylation in leukocytes. There appears to be a direct correlation between the ability of the bacterial peptides to attract the leukocytes and protein carboxymethylation.

Significance to Biomedical Research: Protein carboxymethylation appears to be involved in excitation and secretion of cells and phagocytosis.

Proposed Course of Project: The role of protein carboxymethylation in the pituitary and salivary glands, and blood elements in secretion and chemotaxis will be studied.

Publications:

Diliberto, E.J., Jr., and Axelrod, J.: Regional and Subcellular Distribution of Protein Carboxymethylase in Brain and Other Tissues. J. Neurochem. 26: 1159-1165, 1976.

Diliberto, E.J., Jr., Viveros, O.H., and Axelrod, J.: Subcellular Distribution of Protein Carboxymethylase and its Endogenous Substrates in the Adrenal Medulla: Possible Role in Excitation-Secretion Coupling. Proc. Nat. Acad. Sci. USA 73: 941-944, 1976.

Diliberto, E.J., Jr., Axelrod, J., and Chaiken, I.M.: Effects of Ligands on Enzymic Carboxymethylation of Neurophysins. Biochem. Biophys. Res. Comm. 73: 1063-1067, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00424-02 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biologically Active Peptides in the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. Brownstein, Medical Officer (Research) LCS NIMH
J. Schaeffer, Staff Fellow LCS NIMH

Other: H. Gainer, Section Chief BB NICHHD
M. Palkovits, Guest Worker LCS NIMH
S. Leeman, Asst. Prof. Physiol.; Harvard Medical School
E. Mroz, Post Doctoral Fellow, Harvard Medical School
D Krieger, Prof. Medicine, Mt. Sinai School of Medicine, New York
A. Liotta, Dept. Med., Mt. Sinai School of Medicine, N. Y.
E. Zimmerman, Assoc. Prof. Med., Columbia Univ., New York
A. Robinson, Prof. Medicine, Univ. Pittsburgh, Pittsburgh, Pa.

COOPERATING UNITS (if any)
Harvard Medical School; Mt. Sinai School of Medicine; Columbia University; University of Pittsburgh

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The two neurophysin "carrier" proteins which are made by cells in the supraoptic nucleus of the hypothalamus are synthesized as parts of larger precursor molecules.

Thyrotropin releasing hormone (TRH) is present in high concentrations in the hypothalamus. It can be released from hypothalamic synaptosomes by depolarization with K^+ in the presence, but not the absence, of Ca^{++}. TRH is also found in the retina. The level of TRH increases when animals are exposed to light and decreases when they are placed in the dark.

ACTH has been shown to be present throughout the brain. It is found in particularly high concentrations in limbic structures, where its levels are not altered by hypophysectomy. The regional distribution of ß lipotropin, ß endorphin, neurotensin, substance P, TRH, somatostatin, and glutamic acid decarboxylase have been studied.

Project Description:

Objectives: To locate specific groups of neurons in the central nervous system which synthesize and secrete peptides; to determine the biochemical mechanisms underlying the synthesis of peptides by neurosecretory cells; to study interactions between peptidergic and non-peptidergic neurons.

Major Findings: The two neurophysin "carrier" proteins which are made by cells in the supraoptic nucleus of the hypothalamus are synthesized as parts of larger precursor molecules. These precursors are first processed into smaller, intermediate-sized proteins, and the intermediates give rise to the neurophysins. The two hormones of the posterior pituitary--vasopressin and oxytocine--are likely to be synthesized as parts of the neurophysin precursor proteins.

Thyrotropin releasing hormone (TRH) is present in high concentrations in the hypothalamus. It can be released from hypothalamic synaptosomes by depolarization with K^+ in the presence, but not in the absence of, Ca^{++}. It is also released from hypothalamic synaptosomes by dopamine.

TRH is present in a clone of cells derived from retina. TRH is also found in the retina itself. The level of TRH increases when animals are exposed to light and decreases when they are placed in the dark. Thus, TRH may play an important part in the functioning of the eye.

ACTH has been shown to be present throughout the brain and probably has an important role in the CNS distinct from its role in the periphery. It is found in particularly high concentrations in limbic structures, where its levels are not altered by hypophysectomy. ß-lipotropin and ß-endorphin were found in brains of cows and sheep. The distribution of these two proteins was fairly similar. On the other hand, ACTH (which seems to be synthesized from the same precursor protein as ß-lipotropin and ß-endorphin) has a somewhat different regional distribution. This hints at the importance of peptidase activity in determining a cell's hormonal character.

The regional distribution of neurotensin, substance P, TRH, somatostatin, and glutamic acid decarboxylase have been studied as well.

Significance to Biomedical Research: Peptides secreted by neurons in the CNS appear to act not only as factors which control the anterior pituitary but also as agents (neurotransmitters) which allow nerve cells to interact with one another. Thus, it is important to develop methods for studying these compounds.

Proposed Course of Project: The effect of endocrinological, surgical and pharmacological manipulations on peptide secretion will be studied.

Publications:

Brownstein, M.J.: Biogenic Amine Content of the Hypothalamic Nuclei. In Stumpf, W.E. and Grant, L.D. (eds.): Anatomical Neuroendocrinology. Basel, S. Karger, 1975, pp. 393-396.

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Brownstein, M.J., Mroz, E.A., Kizer, J.S., Palkovits, M. and Leeman, S.E.: Regional distribution of substance P in the brain of the rat. Brain Res. 116: 299-305, 1976.

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Palkovits, M., Brownstein, M.J., Arimura, A., Sato, H., Schally, A.V. and Kizer, J.S.: Somatostatin content of the hypothalamic ventromedial and arcuate nuclei and the circumventricular organs in the rat. Brain Res. 109: 430-434, 1976.

Palkovits, M., Brownstein, M.J., Kizer, J.S., Saavedra, J.M. and Kopin, I.J.: Effect of Stress on Serotonin and Tryptophan Hydroxylase Activity of Brain Nuclei. In Usdin, E., Kvetnansky, R. and Kopin, I.J. (eds): Catecholamines and Stress. New York, Pergamon Press, 1976, pp. 51-58.

Brownstein, M.J.: Hormonal Regulation of the Synthesis and Metabolism of Neurotransmitters. In Naftolin, F., Ryan, K.J. and Davies, J. (eds.): Subcellular Mechanisms in Reproductive Neuroendocrinology. Amsterdam, Elsevier Scientific Publishing Co., 1976, pp. 185-192.

Tappaz, M.L., Brownstein, M.J. and Palkovits, M.: Distribution of glutamate decarboxylase in discrete brain nuclei. Brain Res. 108: 371-379, 1976.

Brownstein, M.J.: Biologically Active Peptides in the Mammalian Central Nervous System. In Gainer, H. (ed.): Peptides in Neurobiology. New York, Plenum Press, 1977, pp. 145-170.

Brownstein, M.J., Arimura, A., Fernandez-Durango, R., Schally, A.V., Palkovits, M. and Kizer, J.S.: The effect of hypothalamic deafferentation on somatostatin-like activity in the rat brain. Endocrinology 100: 246-249, 1977.

Brownstein, M.J., Mroz, E.A., Tappaz, M.L. and Leeman, S.E.: On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. Brain Res. 131: , 1977.

Gainer, H., Sarne, Y. and Brownstein, M.J.: Neurophysin biosynthesis: Conversion of a putative precursor during axonal transport. Science 195: 1354-1356, 1977.

Gainer, H., Sarne, Y. and Brownstein, M.J.: Biosynthesis and axonal transport of rat neurohypophysial proteins and peptides. J. Cell. Biol. 73: 366-381, 1977.

Krieger, D.T., Liotta, A. and Brownstein, J.J.: Corticotropin releasing factor distribution in normal and Brattleboro rat brain, and effect of deafferentation, hypophysectomy and steroid treatment in normal animals. Endocrinology 100: 227-237, 1977.

Krieger, D.T., Liotta, A., and Brownstein, J.J.: Presence of corticotropin in brain of normal and hypophsectomized rats. Proc. Nat. Acad. Sci. USA 74: 648-652, 1977.

Liebschutz, J., Airoldi, L., Brownstein, M.J., Chinn, N.G. and Wurtman, R.J.: Regional distribution of endogenous and parenteral glutamate, aspartate and glutamine in rat brain. Biochem. Pharm. 26: 443-446, 1977.

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Tappaz, M.L., Brownstein, M.J. and Kopin, I.J.: Glutamate decarboxylase (GAD) and γ-aminobutyric acid (GABA) in discrete nuclei of hypothalamus and substantia nigra. Brain Res. 125: 109-122, 1977.

Brownstein, M.J.: Neurotransmitters and hypothalamic hormones in the central nervous system. Fed. Proc. 36: 1960-1963, 1977.

Tappaz, M.L. and Brownstein, M.J.: Origin of glutamate-decarboxylase (GAD)-containing cells in discrete hypothalamic nuclei. Brain Res. 129: , 1977.

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Krieger, D.T., Liotta, A., Suda, T., Palkovits, M. and Brownstein, M.J.: Presence of immunoassayable ß-lipotropin in bovine brain and spinal cord: Lack of concordance with ACTH concentrations. Biochem. and Biophys. Res. Comm. 76: 930-936, 1977.

Schaeffer, J.M., Brownstein, M.J. and Axelrod, J.: Thyrotropin-releasing hormone-like material in the rat retina: Changes due to environmental lighting. Proc. Nat. Acad. Sci. USA, in press. (August)

Brownstein, M.J. and Gainer, H.: Neurophysin biosynthesis in normal rats and in rats with hereditary diabetes insipidus. Proc. Nat. Acad. Sci. USA, in press. (September)

Brownstein, M.J., Robinson, A., and Gainer, H.: Immunological identification of rat neurophysin precursors. Nature, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 MH 00425-01 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Catecholamines and Experimental Hypertension

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Saavedra, Visiting Scientist	LCS NIMH
Other:	H. Grobecker, Guest Worker	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the role of the catecholamines in the central and peripheral nervous system in experimental hypertension.

There is an elevation of the adrenaline-forming enzyme (PNMT) in young (14-week-old) spontaneous hypertensive rats but not in adult animals in which hypertension is fully developed.

A decreased amount of noradrenaline and dopamine ß hydroxylase in selective areas of the anterior and posterior hypothalamus in spontaneous hypertensive and DOCA salt animals was found.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of the catecholamines in the central and peripheral nervous system in experimental hypertension.

Methods Employed: Enzymatic, radiometric, microanatomic and pharmacological.

Major Findings: There is an elevation of the adrenaline-forming enzyme (PNMT) in young (14-week-old) spontaneous hypertensive rats but not in adult animals in which hypertension is fully developed.

A decreased amount of noradrenaline and dopamine-β-hydroxylase in selective areas of the anterior and posterior hypothalamus in spontaneous hypertensive and DOCA salt animals was found.

Significance to Biomedical Research: There appears to be a deficiency in noradrenergic neurons in specific areas of the hypothalamus and an increase in adrenaline in the brain stem. These findings might explain the action of antihypertensive drugs (clonidine and aldomet) and implicate adrenaline in the brain stem as one of the causes of hypertension.

Proposed Course of Project: Catecholamines and their enzymes will be examined in specific brain nuclei in various forms of experimental hypertension (sinoaortic, spontaneous, DOCA salt). The effect of PNMT inhibitors on the reduction of blood pressure will be examined.

Publications:

Axelrod, J.: Biochemical and Pharmacological Approaches in the Study of Sympathetic Nerves. In Worden, F.G., Swazey, J.P. and Adelman, G. (eds.): The Neurosciences: Paths of Discovery. Cambridge, Mass., The MIT Press, 1975, pp. 191-208.

Ciaranello, R.D., Wooten, G.F., and Axelrod, J.: Regulation of Dopamine β-Hydroxylase in Rat Adrenal Glands. J. Biolog. Chem. 250: 3204-3211, 1975.

Wooten, G.F., Jacobowitz, D.M., Saavedra, J.M., and Axelrod, J.: Localization of Extraneuronal Dopamine-β-Hydroxylase in Rat Salivary Gland by Immunofluorescence Histochemistry. J. Neurochem. 24: 1107-1110, 1975.

Wooten, G.F., Kopin, I.J., and Axelrod, J.: Effects of Colchicine and Vinblastine on Axonal Transport and Transmitter Release in Sympathetic Nerves. Ann. N.Y. Acad. Sci. 253: 528-534, 1975.

Axelrod, J.: Catecholamines and Hypertension. Clin. Sci. Mol. Med. 51: S415-S421, 1976.

Axelrod, J., Saavedra, J.M., and Usdin, E.: Trace Amines in the Brain. In Usdin, E. and Sandler, M. (eds.): Psychopharmacology Volume 1: Trace Amines and the Brain. New York, M. Dekker, Inc., 1976, pp. 1-20.

Ciaranello, R.D., Wooten, G.F., and Axelrod, J.: Regulation of Rat Adrenal Dopamine β-Hydroxylase. II Receptor Interaction in the Regulation of Enzyme Synthesis and Degradation. Brain Res. 113: 349-362, 1976.

Saavedra, J.M., and Axelrod, J.: Octopamine as a Putative Neurotransmitter. In Costa, E., Giacobini, E. and Paoletti, R. (eds.): Advances in Biochemical Psychopharmacology, Volume 15. New York, Raven Press, 1976, pp. 95-110.

Tallman, J.F., Saavedra, J.M., and Axelrod, J.: A Sensitive Enzymatic-Isotopic Method for the Analysis of Tyramine in Brain and Other Tissues. J. Neurochem. 27: 465-469, 1976.

Tallman, J.F., Saavedra, J.M., and Axelrod, J.: Biosynthesis and Metabolism of Endogenous Tyramine and its Normal Presence in Sympathetic Nerves. J. Pharmacol. Exp. Ther. 199: 216-221, 1976.

Axelrod, J.: Regulation of the Synthesis, Release, and Actions of Catecholamine Neurotransmitters. In Dumont & Nunez (eds.): First European Symposium on Hormones and Cell Regulation. Elsevier/North-Holland Biomedical Press, 1977, pp. 137-155.

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Kebabian, J.S., Saavedra, J.M., and Axelrod, J.: A Sensitive Enzymatic-Radioisotopic Assay for 3,4-Dihydroxyphenylacetic Acid. J. Neurochem. 28: 795-801, 1977.

Saavedra, J.M., Grobecker, H., and Axelrod, J.: Biochemical and Morphologic Study of Catecholamine Metabolism in Spontaneously Hypertensive Rats. Mayo Clin. Proc. 52: 391-394, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00426-01 LCS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Determination, Localization and Formation of Catechol Estrogens in the Brain and Other Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Paul, Guest Worker	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Others:	J. Saavedra, Visiting Scientist	LCS NIMH
	P. Skolnick	LC NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to develop a sensitive assay for measuring catechol estrogen in tissues and to study its localization, formation and actions.

A sensitive and specific enzymatic assay to measure catechol estrogen in tissues has been developed. Using this assay, catechol estrogens were found to be present in brain, pituitary, liver and ovary. The enzyme that converts estrogen to catechol estrogens has been found in the brain. It is localized in the microsomes and requires cytochrome P450. This is the first microsomal P450 enzyme found in brain. Catechol estrogen blocks the stimulation of cyclic AMP in the rat hypothalamus by estrogen.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop a sensitive assay for measuring catechol estrogen in tissues and to study its localization, formation and actions.

Methods Used: Enzymatic, endocrinological.

Major Findings: A sensitive and specific enzymatic assay to measure catechol estrogen in tissues has been developed. Using this assay, catechol estrogens were found to be present in brain, pituitary, liver and ovary. The enzyme that converts estrogen to catechol estrogens has been found in the brain. It is localized in the microsomes and requires cytochrome P_{450}. This is the first microsomal P_{450} enzyme found in brain. Catechol estrogen blocks the stimulation of cyclic AMP in the rat hypothalamus by estrogen.

Significance to Biomedical Research: Catechol estrogens have been found to have powerful endocrinological effects. The ability to measure this hormone in tissues and blood can give important insight of the occurrence and regulation of this compound in health and disease.

Proposed Course of Project: Plasma levels of catechol estrogen in women and men will be determined under various normal and pathological conditions. Brain levels, and blood and other tissue levels of catechol estrogens will be examined after various endocrine manipulations (ovarectomy, adrenalectomy). Relationship between central adrenergic nervous system and estrogen will be studied.

Publications:

Paul, S. and Skolnick, P.: Catechol estrogens inhibit oestrogen elicited accumulation of hypothalamic cyclic AMP suggesting role as endogenous anti-oestrogens. Nature 266: 559-561, 1977.

Paul, S. and Axelrod, J.: Catechol estrogen-forming enzyme in brain: Demonstration of a cytochrome P_{450}-dependent mono-oxygenase. Endocrinology, in press.

Paul, S. and Axelrod, J.: A rapid and sensitive radioenzymatic assay for catechol estrogens in tissues. Life Sciences, in press.

Paul, S. and Axelrod, J.: Catechol estrogens: Presence in brain and endocrine tissues. Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00401-12 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Formation, Release, Disposition, and Metabolism of Neurotransmitters in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	H. Grobecker	Guest Worker	LCS NIMH
	R. Kvetnansky	Visiting Scientist	LCS NIMH
	G. Maengwyn-Davies	Guest Worker	LCS NIMH
	Z. Poddubiuk	Guest Worker	LCS NIMH
	M. Tappaz	Guest Worker	LCS NIMH
	N. B. Thoa	Pharmacologist	LCS NIMH
	Y. Tizabi	Guest Worker	LCS NIMH
	M. Weinstock	Visiting Scientist	LCS NIMH
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	A. Zavadil	Research Associate	LCS NIMH

COOPERATING UNITS (if any)
Section on Pharmacology
Section on Histopharmacology

LAB/BRANCH
Laboratory of Clinical Science

SECTION
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NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.8	1.2

CHECK APPROPRIATE BOX(ES)

[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The continued objectives of this project are to define the biochemical mechanisms controlling the synthesis, storage, release, action, and termination of action of neurotransmitters and to determine how genetic factors, environmental stimuli, or drugs influence these control mechanisms. During the last year the alterations in release of catecholamines from the adrenal medulla and sympathetic nerves into plasma in genetic hypertension, after various drugs, and during stress have been studied in intact rats. In vitro the influence of presynaptic receptors on release has been examined in cultured sympathetic ganglia.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Plasma levels of catecholamines are extremely labile because they are rapidly removed from the circulation and are released in response to a wide variety of stimuli. A technique has been developed to obtain blood samples from awake undisturbed rats and it has been shown that plasma levels of norepinephrine (NE) and epinephrine (EPI) are lowest during sleep, increase slightly in awake animals, and are increased by even such mild stimuli as transfer of the cage to another room. Handling of rats further increases plasma levels of the catecholamines while forced immobilization produces striking increases in plasma catecholamine content. The spinal shock which attends decapitation produces massive sympatho-adrenal medullary discharge with huge elevation of the amines, particularly of EPI, in plasma. Anesthetic agents markedly depress decapitation-induced catecholamine discharge, while forced immobilization enhances almost three-fold the catecholamine release attending decapitation. In mice exposed to attack, decapitation-induced increases in plasma catecholamines are also enhanced. These results suggest that the spinal mechanism causing sympatho-adrenal discharge are modulated by neuronal impulses from the brain and may be variably responsive to stimuli resulting in activation of the sympathetic nervous system.

Genetic differences in sympathetic responsivity are apparent in spontaneously hypertensive (SHR) rats. In SHR rats, release of catecholamines into plasma produced by several noxious stimuli, such as immobilization, foot shock, and decapitation, is greater than in normotensive control animals of the same (WKY) strain.

Administration of drugs which inhibit metabolism of catecholamines by monoamine oxidase (MAO) or catechol-O-methyl-transferase (COMT) or which inhibit their uptake into sympathetic nerves, produce only minor changes in plasma catecholamine levels. Combined administration of a COMT inhibitor with a MAO inhibitor or a blocker of uptake, increases catecholamine levels in plasma more significantly. Striking increases in catecholamine levels are found, however, when there is a centrally mediated stimulation of catecholamine release (e.g., by cocaine) and COMT has been inhibited.

Administration of oxytremorine produces tremor in rats which is partly reversed by β-adrenergic blocking agents. Oxytremorine causes a central mediated increase in catecholamine release which appears to augment the tremor which is produced by an action in the central nervous system, presumably by an action on cholinergic (muscarinic-type) receptors.

A new adrenergic blocking agent (EMO 21192) has been found to act more specifically on the adrenal medullary secretion of EPI than does guanethidine, which has its major action on sympathetic nerves. The mode of action of EMO 21192 is being studied.

Accumulation of NE in ligated sympathetic axons has been shown to be a consequence of synthesis of the catecholamine rather than axonal transport, so that calculations of the rate of axonal transport are best done using the enzymes (e.g., dopamine-β-hydroxylase) rather than the catecholamine.

The role of various neurotransmitters and pathways in the central nervous system which modulate responsivity of the sympathetic nervous system are now under investigation. The influence of genetic factors, environmental stimuli, hormones and drugs on these processes may explain the etiology of diverse disorders such as hypertension, hysteria, essential tremor, etc., as well as 'highs' and 'lows' in drug abusers and in naturally occurring disorders of affect. Studies in which plasma catecholamines are used as an index of sympatho-adrenal discharge will be combined with studies of the central control of the autonomic nervous system (MH 00402-05 LCS) and complement clinical studies (MH 00403-04 LCS).

PUBLICATIONS:

Grobecker, H., Roizen, M.F., and Kopin, I.J.: Effect of tyramine and guanethidine on dopamine-β-hydroxylase activity and norepinephrine concentrations in vesicular fraction of the heart and plasma of rats. Life Sci. 20: 1009-1016, 1977.

Thoa, N.B., Tizabi, Y., Kopin, I.J., and Maengwyn-Davies, G.D.: Alterations of mouse adrenal medullary catecholamines and enzymes in response to attack: Effect of pre- and post-treatment with phenobarbital. Psychopharmacology 51: 53-57, 1976.

Tizabi, Y., Kopin, I.J., Maengwyn-Davies, G.D., and Thoa, N.B.: Attack-induced changes in response to decapitation of plasma catecholamines of victim mice. Psychopharmacol. Commun. 2: 391-402, 1976.

Roizen, M.F., Moss, J., Henry, D., Weise, V., and Kopin, I.J.: Effect of general anesthetics on handling- and decapitation-induced increases in sympathoadrenal discharge. J. Pharmacol. Exp. Ther. (In press.)

Wooten, G.F., Weise, V., and Kopin, I.J.: Norepinephrine synthesis contributes to the accumulation of catecholamines after ligation of rat sciatic nerves. Brain Res. (In press.)

Kopin, I.J.: Noradrenergic Neurotransmission. In Lipton, M.A., DiMascio, A., and Killam, K.F. (Eds.): Psychopharmacology: A Generation of Progress. New York, Raven Press, 1977. (In press.)

Popper, C.W., Chiueh, C.C., and Kopin, I.J.: Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization, and after decapitation. J. Pharmacol. Exp. Ther. (In press.)

Kopin, I.J.: The role of calcium in neurosecretion. The Proceedings of the 10th Congress of the Collegium Internationale Neuro-Psychopharmacoligicum, Quebec, Canada, 1976. (In press.)

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00402-05 LCS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

CNS Regulation of Autonomic and Endocrine Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. J. Kopin	Chief, LCS	LCS NIMH
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	C. L. Sun	Visiting Fellow	LCS NIMH
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	N. B. Thoa	Pharmacologist	LCS NIMH
	Z. Poddubiuk	Guest Worker	LCS NIMH
	M. Weinstock	Visiting Scientist	LCS NIMH

COOPERATING UNITS (if any)

Section on Pharmacology
Section on Histopharmacology

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to define the areas, pathways, and neurotransmitter systems in the central nervous system which control endocrine, metabolic, and autonomic responses to various types of stress, changes in blood glucose or oxygen tension, hormonal state, body temperature, or other factors.

PHS-6040

PROJECT DESCRIPTION:

Neurotransmitters and their biosynthetic enzymes are assayed by microtechniques and may be examined in small, specific areas in the central nervous system. Lesions produced by substances toxic to particular types of neurones, destruction by electrocoagulation of specific areas of brain or cutting of neuronal tracts connecting different areas produces alterations in levels of the neurotransmitters or enzymes in specific areas and interferes with physiologic responses. By examining these alterations and studying the effects of drugs on changes produced in endocrine, cardiovascular, or metabolic systems, the role of the central nervous system in their control can be determined.

Anesthesia with ether or cyclopropane elevates levels of serotonin and catecholamines in the amygdala, substantia nigra, or nucleus centralis superior, but only cyclopropane elevates serotonin levels in the raphe nucleus. Immobilization stress appears to produce opposite changes. Levels of serotonin are decreased in the amygdala, suprachiasmic nucleus, cingulate cortex, median eminence and dorsal raphes nucleus. With repeated immobilization levels of tryptophan hydroxylase appear to be increased in the latter nucleus.

Glutamic acid decarboxylase and γ-aminobutyric acid (GABA) are also localized to specific areas of the hypothalamus, substantia nigra, and spinal cord. The role of GABA as an inhibitory neurotransmitter in these areas is being studied by use of drugs which mimic or block GABAergic transmission in the spinal cord (see project Z01 MH 00401-12).

Plasma levels of catecholamines reflect sympathoadrenal medullary activity. These levels are increased markedly during immobilization or after administration of 2-desoxyglucose, which mimics hypoglycemia. These procedures also result in secretion of corticosterone from the rat adrenal cortex, presumably as a result of enhanced release of ACTH from the pituitary. Complete hypothalamic deafferentation markedly diminishes the increases catecholamine and corticosterone produced by either of these procedures. Partial deafferentation of the hypothalamus by lateral cuts diminishes the adrenal control responses. Anterior deafferentation increases slightly the corticosterone response to immobilization but does not appear to affect the response to 2-desoxyglucose. Posterior cuts prevent the catecholamine responses to immobilization but do not appear to affect the responses to 2-desoxyglucose. These results suggest that the responses to 2-desoxyglucose and immobilization arise outside the hypothalamus and result in discharge of ACTH by both anterior and lateral pathways. The discharge of catecholamines produced by

immobilization appears to involve pathways which pass through the hypothalamus and emerge posteriorly. Stimulation of the posterior hypothalamic areas inhibits release of catecholamines. Present studies are designed to determine which neurotransmitters may be involved in controlling these responses.

PUBLICATIONS:

Palkovits, M., Brownstein, M., Kizer, J.S., Saavedra, J.M., and Kopin, I.J.: Effect of Stress on Serotonin and Tryptophan Hydroxylase Activity of Brain Nuclei. In Usdin, E., Kvetnansky, R., and Kopin, I.J. (Eds.): Effect of Stress on Serotonin and Tryptophan Hydroxylase Activity of Brain Nuclei. New York, Pergamon Press, Inc., 1976, pp. 51-58.

Roizen, M.F., Kopin, I.J., Thoa, N.B., Zivin, J., Muth, E.A., and Jacobowitz, D.M.: The effect of two anesthetic agents on norepinephrine and dopamine in discrete brain nuclei, fiber tracts, and terminal regions of the rat. Brain Res. 110: 515-522, 1976.

Tappaz, M.L., Zivin, J.A., and Kopin, I.J.: Intraspinal glutamic decarboxylase distribution after transection of the cord at the thoracic level. Brain Res. 111: 220-223, 1976.

Tappaz, M.L., Brownstein, M.J., and Kopin, I.J.: Glutamate decarboxylase (GAD) and γ-aminobutyric acid (GABA) in discrete nuclei of hypothalamus and substantia nigra. Brain Res. 125: 109-121, 1977.

Kopin, I.J.: Mode of Action of: Antidepressants and Central Stimulants. In van Praag, H.M. (Ed.): Handbook of Biological Psychiatry. New York, Marcel Dekker Inc., 1977, Vol. II. (In press.)

Lamprecht, F., Richardson, J.S., Williams, R.B., and Kopin, I.J.: 6-Hydroxydopamine destruction of central adrenergic neurones prevents or reverses developing DOCA-salt hypertension in rats. J. Neural Transm. 40: 149-158, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00403-04 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Control of Catecholamines and DBH in Human Plasma and CSF

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. J. Kopin	Chief, LCS	LCS NIMH
	C. R. Lake	Staff Psychiatrist	LCS NIMH
OTHER:	V. K. Weise	Chemist	LCS NIMH
	F. H. Foppen	Guest Worker	LCS NIMH
	M. H. Ebert	Chief, Section on Exp. Therapeutics	LCS NIMH
	M. D. Coleman	Nephrologist, Holt Krock Med. Clin.,	Fort Smith, Ark.
	D. Krieger	Prof. Med., Mt. Sinai School of Med.,	New York
	J. H. Wood	Neurologist	SN NIH

COOPERATING UNITS (if any)
Section on Experimental Therapeutics
Holt Krock Medical Clinic, Fort Smith, Arkansas
Department of Medicine, Mt. Sinai Hospital, New York, N.Y.
Surgical Neurology Branch (NINCDS), Bethesda, Maryland

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Medicine

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NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Catecholamines, including dopamine (DA), norepinephrine (NE) and epinephrine (EPI) and their metabolites and dopamine-β-hydroxylase are determined in plasma and cerebrospinal fluid (CSF). Levels of the NE in plasma reflect sympathetic neuronal activity while levels of EPI reflect adrenal medullary secretion. Increases in NE normally elicited by standing and by exertion provide a useful index of sympathetic function and this test is used to study the role of the sympathetics in hypertension, orthostatic hypotension of various types, and the effects of psychoactive drugs. Amines are present in CSF, and their levels vary during the day. Amines in CSF may provide a means of studying drug effects on brain amine metabolism.

PROJECT DESCRIPTION:

A method for assay of catecholamines developed in our laboratory has been applied to study levels of these compounds in plasma and cerebrospinal fluid (CSF). In the reclining position, 20-30 minutes after insertion of a needle into an arm vein, levels of norepinephrine (NE) are low and provide an index of basal sympathetic neuronal activity. In normal subjects the levels double on standing and increase three-fold with exertion. Insulin induced hypoglycemia also increases levels of NE, but there is an even greater increase in plasma levels of epinephrine. Patients with orthostatic hypotension may be divided into three groups. Those with low basal levels of NE which do not increase with standing or exertion, those with normal basal levels of NE with absent response to standing, and those with normal basal levels but with normal or supranormal responses to standing. The first type appear to have degenerated sympathetic nerves while the others have intact sympathetic neurones. The second type of orthostatic hypotension is often attended by signs of disorder of the central nervous system suggesting that the otherwise intact sympathetic nerves cannot be activated. In patients with the third type, the reflex responses appear normal but the effector organ (vascular compartment) seems to be unable to respond normally to the released catecholamine. These patients frequently have a marked tachycardia when the blood pressure falls while others do not have any significant increase in pulse rate.

Dopamine-β-hydroxylase (DBH) levels in plasma may reflect long term changes in sympathetic activity and changes in plasma levels of the enzyme appear to be related most closely to rates of synthesis of the enzyme. Levels of DBH increase slightly on standing, but do not increase with exertion although plasma levels of NE progressively increase with standing and exertion. This suggests that the initial increase in DBH is related to fluid and protein redistribution rather than release of the enzyme from sympathetic nerves. Basal levels of NE in plasma increase with age and higher levels of NE reported in hypertensive subjects may be related to the younger ages of the control subjects.

In certain disease states--Lesch-Nyhan Syndrome and Gilles de la Tourette's disease--there appears to be alterations in both DBH and basal levels of NE.

NE in CSF is not derived from plasma, but there appears to be a relationship between levels of NE in plasma and CSF. The levels of NE in CSF of monkeys varies diurnally. Administration of probenecid increases levels of NE in CSF and plasma suggesting the drug influences amine metabolism as well as transport of acid metabolites.

PUBLICATIONS:

Kopin, I.J., Kaufman, S., Viveros, H., Jacobowitz, D.M., Lake, C.R., Ziegler, M., Lovenberg, W.M., and Goodwin, F.K.: Dopamine-β-hydroxylase: Basic and clinical studies. Ann. Intern. Med. 85: 211-223, 1976.

Lake, C.R. and Ziegler, M.G.: Lesch-Nyhan Syndrome: Low dopamine-β-hydroxylase activity and diminished sympathetic response to stress and posture. Science 196: 905-906, 1977.

Lake, C.R., Ziegler, M.G., Coleman, M.D., and Kopin, I.J.: Age-adjusted plasma norepinephrine levels are similar in normotensive and hypertensive subjects. N. Engl. J. Med. 296: 208-209, 1977.

Lake, C.R., Ziegler, M.G., Eldridge, R., and Murphy, D.L.: Catecholamine metabolism in Gilles de la Tourette's syndrome. Am. J. Psychiatry 134: 257-260, 1977.

Weise, V.K. and Kopin, I.J.: Assay of catecholamines in human plasma: Studies of a single isotope radioenzymatic procedure. Life Sci. 19: 1673-1686, 1976.

Ziegler, M.G., Lake, C.R., and Kopin, I.J.: The sympathetic nervous system defect in primary orthostatic hypotension. N. Engl. J. Med. 296: 293-297, 1977.

Ziegler, M.G., Lake, C.R., Wood, J.H., Brooks, B.R., and Ebert, M.H.: Relationship between norepinephrine in blood and cerebrospinal fluid in the presence of a blood-cerebrospinal fluid barrier for norepinephrine. J. Neurochem. 28: 677-679, 1977.

Ziegler, M.G., Lake, C.R., Wood, J.H., and Ebert, M.H.: Circadian rhythm in cerebrospinal fluid noradrenaline of man and monkey. Nature 264: 656-658, 1976.

Ziegler, M.G., Wood, J.H., Lake, C.R., and Kopin, I.J.: Norepinephrine and 3-methoxy-4-hydroxyphenylglycol gradients in human cerebrospinal fluid. Am. J. Psychiatry 134: 565-568, 1977.

Kopin, I.J.: Catecholamine metabolism (and the biochemical assessment of sympathetic activity. Clin. Endocrinol. Metabol. (In press.)

Lake, C.R., Ziegler, M.G., Coleman, M., and Kopin, I.J.: Lack of correlation of plasma norepinephrine and dopamine-β-hydroxylase in hypertensive and normotensive subjects. Circ. Res. (In press.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00404-06 LCS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Growth Characteristics and Immunological Properties of Neurones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. J. Kopin	Chief, LCS	LCS NIMH
OTHER:	A. Liuzzi	Visiting Scientist	LCS NIMH
	F. G. Foppen	Guest Worker	LCS NIMH
	J. M. Saavedra	Medical Officer	LCS NIMH
	D. Schmechel	Medical Officer	LCS NIMH
	G. Gilad	Visiting Fellow	LCS NIMH

COOPERATING UNITS (if any)

Sections on Pharmacology and Histopharmacology

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Medicine

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

NGF promotes growth of several types of cells in the sympathetic nervous system, but this growth differs from that produced by other means, e.g., hormone treatment. Immunological methods are being developed to attempt to determine the different locations of specific proteins (enzymes) in ganglia or adrenals in which growth has been altered by NGF, dexamethasone, or stress.

PHS-6040

PROJECT DESCRIPTION:

Stimulation by nerve growth factor (NGF) of growth of sympathetic neurones in newborn rats is attended by increased levels of phenylethanolamine-N-methyltransferase (PNMT) in the superior cervical ganglia (SCG). Treatment with dexamethasone also produces increases in PNMT in SCG, but not to the same extent. Dexamethasone stimulated increases in PNMT, however, resulted in a parallel increase in levels of epinephrine (EPI) while that produced by NGF does not. Antibodies to PNMT are being prepared to determine the localization of the PNMT increases produced by NGF and dexamethasone to determine why there appears to be differences in the activity of the enzyme in vivo when it is increased by the different stimulants.

The N-methylated product of dopamine, epinine, and of norepinephrine, epinephrine, are found in the superior cervical ganglion. Since dopamine is a precursor to norepinephrine, it has been claimed that epinine may be the precursor to epinephrine. Studies using deuterated methionine have proven that the rate of formation of epinephrine far exceeds that of epinine and that epinine could not be a major precursor of epinephrine in the SCG.

Levels of serotonin and of tryptophan hydroxylase in the SCG are increased by treatment with NGF. Since 6-OH-dopamine, an adrenergic neurotoxin, does not influence these levels, serotonin cannot be present in the sympathetic nerves and therefore must be present in other NGF stimulated cells.

PUBLICATIONS:

Liuzzi, A., Foppen, F., and Kopin, I.J.: Stimulation and maintenance by nerve growth factor of phenylethanolamine-N-methyltransferase in superior cervical ganglia of adult rats. Brain Res. (In press.)

Liuzzi, A., Foppen, F., Saavedra, J., Levi-Montalcini, R., and Kopin, I.J.: Serotonin in rat superior cervical ganglia and the effects of nerve growth factor and 6-hydroxydopamine. Brain Res. (In press.)

Foppen, F.H., Liuzzi, A., and Kopin, I.J.: Gas chromatographic-mass spectrometric identification of epinephrine in rat superior cervical ganglia and formation in vivo. Experientia 33: 596-598, 1977.

Liuzzi, A., Foppen, F.H., Saavedra, J.M., Jacobowitz, D.M., and Kopin, I.J.: Effect of NGF and dexamethasone on phenylethanolamine-N-methyl transferase (PNMT) activity in neonatal rat superior cervical ganglia. J. Neurochem. 28: 1215-1220, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00376-03 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Morphologic Studies of Central Aminergic and Cholinergic Neurons and Pathways.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
OTHER:	P. D. MacLean	Chief, Lab. Brain Evol. and Behav.	LBEB	NIMH

COOPERATING UNITS (if any)

Laboratory of Brain Evolution and Behavior

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A complete monkey brain atlas of the localization of catecholaminergic neurons (cell bodies and nerve terminals) and serotonin-containing cell bodies has been prepared. From a comparative standpoint it is clear that a striking similarity exists in the localization of catecholamine- and serotonin-containing cell bodies between the monkey and the rat. These and other comparative data indicate that a prototypical pattern of organization of aminergic systems has been retained in the evolution of primates.

Stereotaxic maps of noradrenergic pathways (dorsal and ventral bundles) in the rat brain have also been prepared.

Project Description:

Objectives: Preparation of a topographic atlas for the localization of catecholaminergic neurons and serotonergic cell bodies of the monkey (pygmy marmaset) brain.

Methods Employed: The morphological procedure involves the use of a specific fluorescence histochemical method (Falck and Hillarp) for the demonstration of catecholamines and indoleamines (serotonin) within monoaminergic neurones.

Major Findings: A complete monkey brain atlas of the localization of catecholaminergic neurons (cell bodies and nerve terminals) and serotonin-containing cell bodies has been prepared. From a comparative standpoint it is clear that a striking similarity exists in the localization of catecholamine- and serotonin-containing cell bodies between the monkey and the rat.

Significance to Biomedical Research and the Program of the Institute: These aminergic maps provide a template by which researchers might probe the brains of larger primates. Because of the small size of the pygmy marmaset, it is a particularly convenient primate to explore behavioral studies following drug injections or stereotaxic manipulations of aminergic systems.

Proposed Course of the Project: The mapping of monoaminergic neurons in the monkey brain is terminated with publication of a brainstem atlas.

Publications:

Jacobowitz, D.M., and MacLean, P.D.: A brainstem atlas of catecholaminergic neurons and serotonergic perikarya in a pygmy primate (Cebuella Pygmaea). J. Comp. Neurol. (In Press) 1977.

Jacobowitz, D.M.: Histochemical and micropunch analysis of aminergic and cholinergic pathways. Collegium Internationale Neuro-Psychopharmacologicum, 10th International Congress, Quebec, 1976 (In Press).

Jacobowitz, D.M.: Monoaminergic pathways in the central nervous system. Psychopharmacology: A Generation of Progress, 1977 (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00378-03 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunofluorescence Study of the Uptake of the Antibody to Dopamine-beta-Hydroxylase in the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D.M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	M.A. Silver	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study was to observe the possible uptake of antibody to dopamine-beta-hydroxylase (ADβH) into central nervous system noradrenergic neurons following stereotaxic intraventricular or intracerebral injection of ADβH. In vivo administration of ADβH resulted in the specific uptake and localization of the antibody-DBH complex only in noradrenergic neurons (fibers and cell bodies). The suggestion that ADβH was taken up by noradrenergic nerve terminals and transported to neural cell bodies via retrograde processes is supported by three lines of evidence: (1) time course studies, (2) unilateral ADβH injection into the hypothalamic dorsal noradrenergic bundle resulted in the labelling of cell bodies in the locus coeruleus, and (3) bilateral transection of this bundle proximal to the injection site prevented the arrival of ADβH into the locus coeruleus cells.

PHS-6040

Project Description:

Objectives: (1) To study a possible retrograde axonal transport of the antibody to dopamine-beta-hydroxylase in noradrenergic neurons of the rat brain. (2) Determine the value of this method for mapping of the localization of noradrenergic projections to various nuclei and other regions of the brain.

Methods Employed: Immunohistochemical localization of the ADBH by an indirect immunofluorescence technique utilizing a fluorescein-labeled antiserum to the host IgG. The antibody to DBH was injected intraventricularly or intracerebrally into the rat brain. The rats were killed at various periods of time.

Major Findings: Intraventricular injection of ADBH results in a selective uptake of the antibody in noradrenergic nerve terminals and is transported via a retrograde process mainly to the locus coeruleus. Some adrenergic cells of the ventral noradrenergic pathway are observed to contain the ADBH. Bilateral transection of the dorsal noradrenergic bundle, which is derived from the locus coeruleus, prevented the retrograde flow to the locus coeruleus. Control injections of pre-immune serum did not result in the labeling of any central neurons. Injection of ADBH into the dorsal bundle - median forebrain bundle resulted in the appearance of the antibody-DBH complex in to locus coeruleus and ventral bundle cell bodies (A1,A2,A5).

Significance to Biomedical Research and the Program of the Institute: The observation that intracerebrally or intraventricularly administered ADBH is selectively taken up by noradrenergic nerve terminals and transported via retrograde processes to the appropriate associated cell bodies provides three distinct findings. (1) This technique offers a rapid, simple procedure for the visualization of central noradrenrgic systems with a combined sensitivity and specificity equal to or greater than previously reported histofluorescnece of immunohistochemical techniques. (2) The observed retrograde transport of ADBH suggests the presence of an endogenous retrograde transport of DBH, similar to that previously reported in the peripheral nervous system by this laboratory. The significance of this finding is as yet unknown. (3) As a practical neuroanatomical tool, the use of specific terminal uptake and retrograde transport of a marker protein, i.e. ADBH, combined with immunochemical sensitivity and specificity permits the elucidation of specific noradrenergic circuitry patterns which has not been possible with previous histochemical procedures.

Proposed Course of Project: The initial phase of this study which demonstrates uptake and retrograde transport of ADBH by central noradrenergic neurons is completed. The next phase of this project will consist of two studies. The first study will investigate the ADBH uptake process utilizing neuropharmacological approaches; the second study will attempt to elucidate specific projections and circuitry patterns of central noradrenergic cell groups subsequent to discrete injections of ADBH into various central structures.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00379-03 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Histochemical and Biochemical Studies of an Isolated Perfused Rat Brain Preparation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS NIMH
OTHER:	H. A. Sloviter	Professor	Univ. of Pa.
	J. M. George	Professor	Ohio State Univ.

COOPERATING UNITS (if any)

University of Pennsylvania, School of Medicine, Department of Neurosurgery; Ohio State University, School of Medicine, Department of Medicine.

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Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) The isolated perfused rat brain preparation consists of the skull of a rat and its contents with practically all extra-cranial tissue removed. This isolated brain preparation is made from a rat which was adequately anesthetized by deep hypothermia without the use of a chemical agent for anesthesia. This avoids possible interference by residual anesthetic agent on subsequent measurements of activities of the brain. In such a preparation the intensity and density of catecholamine fluorescent varicosities and serotonin cell bodies are essentially similar to that observed in the non-perfused brain taken from rats immediately following decapitation. (2) Perfusion of the isolated brain with methadone or morphine resulted in a decrease in the vasopressin content of the supraoptic nucleus.

Project Description:

See last year's project report.

Proposed Course of Project: This study is terminated with publication.

Publication:

Jacobowitz, D.M., Mizawa, T., and Sloviter, H.A.: Histochemical study of biogenic amines in an isolated perfused rat brain. Brain Res. 110: 152-157, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00381-03 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Fluctuations of Vasopressin and Oxytocin in the Pituitary During the Estrous Cycle of the Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PTS:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	W. P. Crowley	Guest Worker	LCS	NIMH
	T. L. O'Donohue	Guest Worker	LCS	NIMH
OTHER:				
	J. M. George	Professor	Ohio State Univ.	

COOPERATING UNITS (if any)

Ohio State Univ., School of Medicine, Dept. of Med.

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Laboratory of Clinical Science

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Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	.4	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pituitary contents of oxytocin and vasopressin were highest on proestrus and estrus and low on metestrus and diestrus. Norepinephrine levels in the paraventricular nucleus were lowest during proestrus and estrus and high during metestrus and diestrus, but did not change in the supraoptic nucleus.

PHS-6040

Project description:

Objectives: To determine levels of oxytocin and vasopressin in the pituitary during the estrous cycle and to correlate these changes with levels of norepinephrine in the magnocellular hypothalamic nuclei.

Methods Employed: (1) Radioimmunoassay for oxytocin and vasopressin. (2) Microdissection of hypothalamic nuclei. (3) Enzymatic-isotopic assay for norepinephrine.

Major Findings: Pituitary contents of oxytocin and vasopressin were highest on proestrus and estrus and lowest on metestrus and diestrus. Norepinephrine levels in the paraventricular nucleus were lowest during proestrus and estrus and highest during metestrus and diestrus, but did not change in the supraoptic nucleus.

Significance to Biomedical Research and the Program of the Institute: This study demonstrates a relationship between reproductive state and levels of posterior pituitary hormones and suggests a role for oxytocin and vasopressin during the estrous cycle.

Proposed Course of Project: Follow-up studies in progress include the effects of ovariectomy and steroid replacement on oxytocin and vasopressin content in the pituitary and on norepinephrine levels and turnover in the magnocellular hypothalamic nuclei.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00382-03 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Mapping of Monoaminergic Neurons of the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D.M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	T.L. O'Donohue	Guest Worker	LCS	NIMH
	W.R. Crowley	Guest Worker	LCS	NIMH
	S.G. Speciale	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

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Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.3	.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study maps, by biochemical means, the ascending projection areas of the A5 noradrenergic cell bodies of the rat brain by the use of a microdissection technique capable of removing specific brain nuclei and a sensitive isotopic assay for norepinephrine and dopamine. Lesion of the A5 cell grouping resulted in a reduction of the norepinephrine concentration in the medial preoptic nucleus, anterior hypothalamus, median eminence, central amygdaloid nucleus, piriform cortex, bed nucleus of the stria terminalis, and caudate nucleus. A reduction in dopamine concentration was observed in the nucleus accumbens, caudate nucleus, and the median eminence. These changes in dopamine suggest a noradrenergic-dopaminergic interaction.

Project Description:

Objectives: To map by biochemical means the ascending projection areas of the A5 noradrenergic cell bodies of the rat brain.

Methods Employed: (1) Discrete stereotaxic unilateral brain lesions were made of the A5 noradrenergic cell group. Animals were sacrificed after 17 days and brains dissected by the micropunch technique (Palkovits). (2) Regions were assayed for norepinephrine and dopamine by a radioenzymatic technique (Henry).

Major Findings: Areas containing a significant reduction in the norepinephrine concentration were: the caudate nucleus, medial preoptic nucleus, anterior hypothalamus, median eminence, central amygdaloid nucleus, piriform cortex, and the bed nucleus of the stria terminalis. Surprisingly, dopamine also showed a reduction in the nucleus accumbens, the caudate nucleus, and the median aminence. These changes in dopamine concentration suggest a noradrenergic-dopaminergic interaction, perhaps either at the terminal region or at the dopaminergic cell bodies (substantia nigra) innervating these regions.

This study also shows that there is a bilateral projection to many of the innervated sites and that no site receives an entire innervation from the A5 noradrenergic cell grouping.

Significance to Biomedical Research and the Program of the Institute: This study represents an investigation into the precise localization of noradrenergic terminals which emanate from a distinct cell grouping contained in the hindbrain and is known to contribute to the ventral noradrenergic axonal pathway which innervates areas of the forebrain, such as the hypothalamus, and is involved in the modulation of various behavioral parameters. The anatomical organization of the catecholaminergic pathways and terminal innervation is basic for future work concerning the functional organization of aminergic neurons in the brain.

Proposed Course of Project: Biochemical mapping of monoaminergic and cholinergic pathways of the brain will continue.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00383-03 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Effects of Stress on Catecholamine Concentration in Discrete Regions of the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D.M. Jacobowitz	Chief, Sect. on Histopharm.	LCS NIMH
	J.A. Moyer	Guest Worker	LCS NIMH
	L.R. Herrenkohl	Associate Professor	Temple Univ.

COOPERATING UNITS (if any)

Temple University, Dept. of Psychology, Phila. Pa.

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Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study examines the effects of stress during pregnancy on catecholamine concentrations in discrete brain areas of the rat.

This study also examines the effects of prenatal stress on catecholamine concentrations in 24 discrete brain areas of rat offspring as adults. Prenatally-stressed male offspring as adults had significantly lower norepinephrine concentrations in the medial preoptic nucleus and median eminence. Prenatally-stressed female offspring had significantly higher norepinephrine concentrations in the entorhinal cortex, higher dopamine concentrations in the arcuate nucleus and lower dopamine in the nucleus accumbens and periventricular nucleus than did normal adult females. These findings correlate with prior findings that prenatal stress feminizes sexual behavior of male offspring as adults and suggest that brain neurochemical alterations may play a role in the behavioral changes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: (1) To study the effects of stress during pregnancy on catecholamines (norepinephrine, dopamine) in individual discrete brain nuclei and other areas. (2) To study the influence of prenatal stress on catecholamine concentration in discrete brain regions of offspring as adults.

Methods Employed: (1) Pregnant rats were stressed on days 14-21 of pregnancy by confinement for one hour in the presence of intense light, 2 times each day. The rats were sacrificed after the last period of stress. (2) Pregnant rats were stressed, as above, and the offspring were reared by their natural mothers, weaned at 21 days of age, and sacrificed at 90 days of age. (3) A microdissection technique for removal of discrete nuclei and other brain regions (Palkovits). (4) Radioisotopic enzymatic assays for NE and DA (Henry).

Major Findings: (1) Prepartal stress selectively reduced NE in the medial preoptic nucleus, the anterior hypothalamus, the median forebrain bundle. In the median eminence, DA was reduced significantly. Prepartal stress selectively increased NE in the nucleus accumbens and DA in the nucleus amygdaloideus corticalis. (2) Prenatally-stressed male offspring as adults had significantly lower NE concentrations in the medial preoptic nucleus and median eminence. Prenatally-stressed female offspring had significantly higher NE concentrations in the entorhinal cortex, higher DA concentrations in the arcuate nucleus, and lower DA in the nucleus accumbens and periventricular nucleus than did normal adult females.

Significance to Biomedical Research and the Program of the Institute: The locations of prepartal stress-induced alterations in CA correlate with brain regions involved in gonadotrophin release (preoptic region, median eminence, amygdala), as well as with neuroendocrine control mechanisms, including sexual behavior (preoptic nucleus, anterior hypothalamus). The locations of CA decreases as a function of prepartal stress overlap with larger brain regions in which CA depletions have been implicated in functional affective disorders. In humans, anxiety and excessive emotional stress complicate pregnancy and birth. The relatively high incidence of certain mental disorders (e.g., depression) when sex steroids and CA fluctuate widely (during diestrous, in the postpartum period and at menopause) suggests an interrelationship between female hormones, catecholamines, and psychological state. Stress during pregnancy, with the corresponding changes in brain monoamines, may set the stage for postpartum depression.

It is suggested that prenatal-stress induced changes in CA concentrations in regions known to be involved with sexual behavior and gonadotropin secretion, reflect an alteration of the CA within nerve terminals in discrete areas (e.g., medial preoptic nucleus and median eminence) rather than changes in regions innervated by an entire monoaminergic system. In this manner, CA changes act as a convenient "marker" for regions that have been affected by prenatal stress. It is further suggested that prenatal stress, in rats, may result in hormonally-induced permanent neurochemical changes that are responsible for feminization of male offspring.

Proposed Course of Project: Studies on stress are being continued.

Publications:

Thoa, N.B., Tizabi, Y., and Jacobowitz, D.M.: The effect of isolation on catecholamine concentration and turnover in discrete areas of the rat brain. Brain Res. (In Pr ess) 1977.

Moyer, J.A., Herrenkohl, L.R., and Jacobowitz, D.M.: Effects of stress during pregnancy on catecholamines in discrete brain regions. Brain Res. 121: 385-393, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00386-02 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of the Brains of Genetically Obese Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D.M. Jacobowitz	Chief, Sect. on Histopharm.	LCS, NIMH
	W.R. Crowley	Guest Worker	LCS, NIMH
OTHER:	J.M. George	Professor	Ohio State Univ.

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pituitaries of genetically obese (Zucker-fatty) rats contained significantly less vasopressin than the pituitaries of the phenotypically normal littermates.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In a prior study we showed that Zucker-fatty rats had a decreased norepinephrine concentration in the paraventricular nucleus. Since the latter nucleus synthesizes vasopressin and oxytocin it was considered of interest to measure the levels of these hormones in individual hypothalamic nuclei of Zucker-fatty rats.

Methods Employed: (1) Microdissection technique for removal of discrete nuclei and other brain regions. (2) Radioimmunoassay for oxytocin and vasopressin.

Major Findings: (1) Pituitaries of the Zucker-fatty rats contained significantly less (41%) vasopressin than the pituitaries of the lean controls. (2) No differences in vasopressin and oxytocin were noted in the paraventricular and supraoptic nuclei.

Significance to Biomedical Research and the Program of the Institute: This study focuses attention on a possible role of vasopressin in consumatory behavior.

Proposed Course of Project: Further studies which include chronic implantation of vasopressin in genetically obese rats is contemplated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 00387-02 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Chromaffin Cells in Chick Embryo Sympathetic Ganglion Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D.M. Jacobowitz	Chief, Sect. on Histopharm.	LCS NIMH
	N.B. Thoa	Guest Worker	LCS NIMH
	L.A. Greene	Professor	Children's Hosp. Med. Ctr., Boston, Mass.

COOPERATING UNITS (if any)

Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts - Section on Medicine

LAB/BRANCH

Laboratory of Clinical Science

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Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project completed with publication of results.

PHS-6040
(Rev. 10-76)

Publication:

Jacobowitz, D.M., Greene, L.A., and Thoa, W.B.: Chromaffin cells in culture. Fogarty International Center Proceedings No. 30. DHEW Publication No. (NIH) 76-942, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00388-01 LCS

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Distribution of Acetylcholine and Cholinergic Enzymes in Discrete Areas of Rat Forebrain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	D. B. Hoover	Guest Worker	LCS	NIMH
	E. A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.1	.7

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[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) By employing a microdissection technique and sensitive radiometric assays, the distributions of acetylcholine (ACh), choline acetyltransferase (CAT), and acetylcholinesterase (AChE) were examined in 60 discrete areas of rat forebrain. A 5-fold range was observed for ACh levels, while that for the cholinergic enzymes was about 100-fold. AChE activity was always higher than that of CAT. ACh levels did not appear to relate to the activity of either enzyme in a simple manner. However, there was a good overall correlation between CAT and AChE activities. Both ACh and the enzymes were concentrated in the striatum. The highest forebrain CAT activity was found in the n. habenulae medialis. One or more of the cholinergic parameters occurred in moderate to high amounts in several limbic structures. These included the n. accumbens, olfactory tubercle, septum, n. tractus diagonalis, piriform and entorhinal cortices, and some thalamic and amygdaloid nuclei. In the hypothalamus, substantial CAT activity was found in the median eminence. Such biochemical measurements should prove valuable in future experiments designed to determine cholinergic function and localize cholinergic pathways.

Project Description:

Objectives: (1) To study the discrete distributions of acetylcholine (ACh), choline acetyltransferase (CAT), and acetylcholinesterase (AChE) in rat forebrain to gain a better understanding of sites where cholinergic neurons are located.

Methods Employed: (1) Rats were killed by microwave irradiation prior to ACh analysis to prevent its rapid, enzymatic hydrolysis. Decapitation was used if enzymes were to be measured. (2) Unfixed tissue was sectioned in a cryostat. Since microwave-fixed tissue sectioned poorly by this method, it was sectioned in a vibratome using an inert flurocarbon liquid (FC-47) to prevent diffusion (Hoover et al.). (3) Discrete forebrain regions were removed from 300 µm coronal brain sections by a microdissection technique (Palkovits). (4) Sensitive radiometric methods were employed to measure ACh (Goldberg and McCaman), CAT (Fonnum), and AChE (Hoover et al.).

Major Findings: (1) Using sensitive radiometric methods, ACh, CAT, and AChE were detected in all of 60 discrete areas dissected from rat forebrain. The range for ACh levels was 5-fold, while that for cholinergic enzymes was about 100-fold. The activity of AChE was always higher than that of CAT. (2) The ratios of ACh:CAT and ACh:AChE did not remain constant from one brain region to another, but the ratios of CAT:AChE were fairly similar, with a correlation coefficient of 0.902 (excluding n. habenulae medialis). (3) Highest levels of ACh were found in the striatum. CAT and AChE were likewise elevated in this region. CAT activity was highest in the n. habenulae medialis, while ACh and AChE were not correspondingly elevated. One or more of the cholinergic parameters occurred in moderate to high amounts in several limbic structures. These included the n. accumbens, olfactory tubercle, septum, n. tractus diagonalis, piriform and entorhinal cortices, and a number of thalamic and amygdaloid nuclei. In the hypothalamus, substantial CAT activity was found in the median eminence. (4) Biochemical measurements of AChE agreed well with histochemical studies. The enzyme assays were more sensitive, detecting AChE where histochemical procedures failed.

Significance to Biomedical Research and the Program of the Institute: This study has provided a detailed biochemical map of the distribution of cholinergic neuron markers in rat forebrain. Reliable histochemical methods are not available for ACh or CAT, but are for AChE. However, AChE is located in both cholinergic and cholinoceptive neurons. Such biochemical studies extend our knowledge of the distribution of cholinergic systems in the brain and, thereby, suggest possible functions for them.

Proposed Course of Project: (1) Cholinergic pathways will be mapped by examining the effects of electrolytic and knife lesions on acetylcholine and the cholinergic enzymes in discrete brain regions. (2) Cholinergic systems will be examined in specific regions to determine what functional role they may play.

Publications:

Jacobowitz, D.M. and Goldberg, A.M.: Determination of acetylcholine in discrete regions of the rat brain. Brain Res. 122: 575-577, 1977.

Hoover, D.B., Muth, E.A., and Jacobowitz, D.M.: A method for sectioning microwave-fixed brain prior to microdissection and acetylcholine analysis Neuroscience Letters, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 00389-01 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Measurement of Catecholamines in Specific Areas of the Brain During the Estrous Cycle of the Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	W. R. Crowley	Guest Worker	LCS	NIMH
	T. L. O'Donohue	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.4	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Norepinephrine levels fluctuated in the medial preoptic, paraventricular and lateral septal nuclei, and in the mesencephalic central gray catecholamine area during the four day estrous cycle of the female rat. Dopamine levels fluctuated in the lateral septum, nucleus of the diagonal band, caudate and median eminence.

PHS-6040

Project Description:

Objectives: To determine whether catecholamine concentrations change in discrete brain nuclei during the estrous cycle.

Methods Employed: (1) The microdissection technique to remove individual brain nuclei. (2) The enzymatic-isotopic microassay for catecholamines.

Major Findings: Norepinephrine decreased in the medial preoptic and paraventricular nuclei on proestrus and increased on metestrus. Norepinephrine increased on metestrus in the lateral septum and central gray catecholamine area. Dopamine decreased on proestrus in the caudate and rose on diestrus. Dopamine rose on diestrus in the lateral septum and on metestrus in the nucleus of the diagonal band. Dopamine increased from proestrus to estrus in the median eminence.

Significance to Biomedical Research and the Program of the Institute: The present study helps to delineate (1) how ovarian hormones interact with central neurotransmitter systems to regulate gonadotropin secretion, ovulation, and mating behavior and (2) the neuroanatomical loci for this control.

Proposed Course of Project: Further studies on the effects of ovarian hormones on catecholamine turnover are in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00390-01 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Sex Differences in Catecholamine Concentration in Discrete Regions of the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	W. R. Crowley	Guest Worker	LCS	NIMH
	T. L. O'Donohue	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.4	.4

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[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adult male rats had higher levels of norpinephrine and dopamine in several hypothalamic and forebrain nuclei. Castration of males on day 1 of life reduced adult levels of dopamine to female levels in the arcuate and diagonal band nuclei and administration of testosterone to females on day 4 of life raised dopamine levels in adulthood in these two regions. The neonatal androgen manipulations did not affect the sex differences in norepinephrine.

PHS-6040
(Rev. 10-76)

Project description:

Objectives: To determine whether sex differences exist in catecholamine concentration in discrete brain nuclei and whether such differences can be affected by manipulating neonatal androgen.

Methods Employed: (1) Microdissection technique to remove individual brain nuclei. (2) Enzymatic-isotopic microassay for catecholamines.

Major Findings: Males had higher levels of norepinephrine than females in the preoptic-suprachiasmatic, arcuate, periventricular, paraventricular nuclei, and in the median eminence. These differences were not affected by either castration of males on day 1 of life or by administration of testosterone to females on day 4 of life. Males had higher levels of dopamine than females in the arcuate, caudate, median eminence and diagonal band nuclei. Neonatal castration of males lowered dopamine in the arcuate and diagonal band, and neonatal administration of testosterone to females elevated dopamine in these nuclei.

Significance to Biomedical Research and the Program of the Institute: These studies indicate that catecholamines may be permanently altered in some areas during the androgen-dependent processes of sexual differentiation, and these changes may underly, in part, the sex differences in behavior, gonadotropin secretion, and sensitivity to hormones.

Proposed Course of Project: Terminated with a manuscript submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00391-01 LCS

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neurochemical and Anatomical Studies of GABAergic Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	D. M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Z. Gottesfeld	Guest Worker	LCS	NIMH
	V. J. Massari	Guest Worker	LCS	NIMH
	E. A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.1	.4

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[ ] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [X] (c) NEITHER

[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work describes the distribution of glutamic acid decarboxylase (GAD), a GABA synthesizing enzyme, in the brain. Evidence is also presented which suggests that the stria medullaris (SM), a major fiber system which connects fore- and hindbrain, contains GABAergic afferents to the lateral habenula (LHb). Following SM lesions, it was found that in the LHb GAD activity was decreased by 57% and the uptake of GABA by synaptosomes was reduced by 62%. The medial habenula (MHb) was not affected. The LHb and MHb manifest a differential uptake capacity for GABA and glutamate, showing a higher accumulation by LHb. GABA uptake is predominantly by synaptosomes compared to glial uptake, as shown by drug studies. Part of the GABAergic projection to the LHb appears to originate from the globus pallidus and interpeduncular nuclei.

Project description:

Objectives: To map the distribution of GABAergic neurons in discrete brain regions. (2) To identify the source and projection sites of GABAergic pathways. (3) To study the interaction of GABA with other neurotransmitters in the brain.

Methods Employed: (1) GABAergic neurons were identified by their marker enzyme glutamic acid decarboxylase (GAD). This was measured by a radiometric assay (Fonnum) of discrete nuclei and other regions removed by microdissection (Palkovits).

Major Findings: (1) GAD is unevenly distributed in the brain and shows a high activity in regions associated with the basal ganglia, hypothalamic nuclei, and limbic system. (2) The work presents evidence, based on discrete brain lesions, that (a) the stria medullaris, a major fiber connection between the fore- and midbrain limbic structures, contains GABAergic afferents to the lateral habenula (LHb); (b) the source of these fibers, at least in part, is located in the basal ganglia structures such as globus pallidus and entopeduncular nuclei; (c) glutamic acid may be an excitatory neurotransmitter in the habenular nuclei.

Significance to Biomedical Research and the Program of the Institute: The habenular nuclei have been implicated in a number of behavioral and physiological manifestations, and have been considered as a relay station between fore- and midbrain limbic structures. The identity of neurotransmitters projecting to and emanating from this region to other target areas in the brain may provide firstly, basic information on the interaction amongst neurotransmitters in the CNS and secondly, their involvement in brain function and defined behavioral patterns controlled by discrete brain regions.

Proposed Course of Project: Studies on the interaction of GABAergic with other neurotransmitter-specific neurons in discrete brain regions are in progress.

Publications:

Massari, V.J., Gottesfeld, Z., and Jacobowitz, D.M.: Distribution of glutamic acid decarboxylase in certain rhombencephalic and thalamic nuclei of the rat. Brain Res. 118: 147-151, 1976.

Gottesfeld, Z. and Jacobowitz, D.M.: Neurochemical and anatomical studies of GABAergic neurons, Chapter 8 in "Interactions Amongst Putative Neurotransmitters". Raven Press, Pub. (in press), 1977.

Gottesfeld, Z., Massari, V.J., Muth, E.A., and Jacobowitz, D.M.: A possible pathway containing GABAergic afferents to the lateral habenula. Brain Res. (in press), 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00351-03 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	C. R. Lake	Staff Psychiatrist	LCS NIMH
	A. Zavadil	Staff Fellow	LCS NIMH
	M. Perlow	Staff Fellow	LNP NINCDS
	R. Kartzinel	Guest Worker	LCS NIMH
	E. Gordon	Head, Unit on Clinical Biochemistry	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Neuropharmacology, IR, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.5	1.2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to carry out pharmacokinetic studies of psychoactive drugs. The technique of giving probenecid intravenously to rapidly block transport of acid metabolites of dopamine and serotonin has been further explored this year in primates at doses ranging from 40-160 mg/Kg. Data derived from the lateral ventricle and lumbar space of the rhesus monkey suggest that quantitative data can be derived from lumbar CSF metabolite messurements in patients if CSF probenecid level is taken into account. Stable isotope techniques for studying brain amine metabolism have been further developed this year. Studies were concluded in rhesus monkeys on the feasibility of labelling CSF homovanillic acid (HVA) originating from brain dopamine metabolism with deuterated methionine. A quantitative estimate of HVA turnover in the lateral ventricle was calculated. The infusion of deuterium labelled HVA in patients was combined with an intravenous probenecid infusion to arrive at an estimate of central nervous system dopamine turnover in man. Diurnal studies of levels of catecholamine metabolites in the lateral ventricle of primates was completed.

Project Description:

Objectives: 1. To develop techniques for studying kinetic parameters of central nervous system monoamine metabolism in man. 2. To carry out pharmacokinetic studies of psychoactive drugs. 3. To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed:

1. Biochemical methods: Flourometric, gas chromatographic, and mass sprectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radioimmunoassay techniques are used for assay of drugs in body fluids (probenecid and d-amphetamine).

2. Physiological methods: As the project progresses, an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical in vivo experiments on brain metabolism, a small primate metabolic laboratory has been developed with the capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired rhesus monkeys for days or weeks at a time.

Major Findings:

Dr. Ebert and Dr. Kartzinel pursued further studies of transport blockade of acidic CSF amine metabolites by intravenous probenecid by studying the effect of 40, 80, 160 mg/Kg of probenecid on homovanillic acid (HVA) and five-hydroxy-indole-acetic acid (5HIAA) in lateral ventricle and lumbar space of the rhesus macaque. We have used 40 mg/Kg doses intravenously in clinical metabolic studies, and 160 mg/Kg produces essentially complete blockade of transport in rat brain. Maximum probenecid concentrations were attained 2 hours earlier and were 40% higher in the ventricle than in the lumbar space. The elimination half life of probenecid showed a significant increase with the dose of probenecid. The probenecid-induced rise in HVA and 5HIAA levels showed a significant elevation with increasing dose of probenecid in lumbar CSF, but not with each dose of probenecid in ventricular CSF. Significant positive correlations were found between CSF levels of probenecid, HVA, and 5HIAA in both ventricular and lumbar CSF.

Drs. Ebert, Kopin, and Perlow have continued experiments begun last year using stable isotopes to label the synthesis of the major dopamine metabolite, HVA, appearing in brain and CSF. The kinetics of the isotopic species can be used to estimate turnover rates. We have shown that methionine labelled with deuterium on the methyl group (Me-CD_3), 50 mg/Kg, administered intravenously to rats, results in a progressive increase in HVA-CD_3, and that this method can be used to measure HVA formation. In

primates, Me-CD_3 administered intravenously appears in lateral ventricle CSF. The relative enrichment of Me with CD_3 progressively decreases while that of HVA increases until it reaches that of the Me. After the relative enrichment of HVA reaches this peak, it declines more slowly than that of Me. This precursor-product relationship can be used to estimate the turnover constant for HVA in CSF. In similar experiments with ^{18}O to label dopamine synthesis, the kinetics of ^{18}O-HVA appearance and disappearance in CSF can be used to estimate dopamine turnover.

Drs. Kopin, Ebert, Gordon, and Zavadil have pursued studies of the feasibility of using deuterium labelled HVA (d_2-HVA) in an intravenous bolus, to determine turnover rates and volume of distribution of HVA in patients. These studies have posed difficult problems in analytical chemistry which now appear to be overcome. HVA disappears from circulation with a half life of approximately 40 minutes. Synthesis rate for hva calculated from the K_E of d_2-HVA and its volume of distribution agree favorably with urinary excretion rates of HVA. When the d_2 HVA infusion is followed after 75 minutes by an infusion of probenecid (40 mg/Kg), the excretion rate of d_2HVA decreases by 70-90%. From the altered kinetics of plasma HVA following probenecid, calculations can be made for the synthesis rate of a probenecid sensitive HVA compartment (presumably this is primarily the central nervous system). It appears that approximately 50% of HVA production originates from this compartment.

Drs. Perlow, Gordon, Lake, and Ebert concluded several studies this year of diurnal changes in several amine metabolites in lateral ventricle CSF. There is a clear diurnal variation in CSF norepinephrine levels from 125 to 225 pg/ml. The nadir occurs at 6:00 a.m., and the apex at 4:00 p.m. MHPG and VMA, the two major metabolites of norepinephrine, do not show a significant variation in the same samples of CSF. This offers supporting evidence for the concept that these metabolites are rapidly cleared from the central nervous system, and do not accurately reflect changes in brain norepinephrine production rates. Homovanillic acid, the major metabolite of dopamine, also has a significant diurnal variation in the lateral ventricle CSF in phase with the norepinephrine rhythm, although the absolute magnitude of the change is considerably smaller.

Studies of the pharmacokinetics of d-amphetamine in hyperactive children continued this year in collaboration with the Biological Psychiatry Branch. Plasma levels of amphetamine peak at approximately 60 ng/ml between 3-4 hours after a single oral dose of 0.5 mg/kg, and subsequently fall with an apparent half life of 6 hours. There is a significant response in behavioral pathology, but not in motor activity to a single oral dose of amphetamine in comparison to placebo. Behavioral response is maximal between 2-3 hours after drug administration while plasma amphetamine level is rising rapidly. Studies of cortical average evoked response in relation to plasma amphetamine level and behavioral response are in progress with Dr. Buchsbaum. Initial data analysis in these studies demonstrates a highly significant correlation between

a decrease in variance of average evoked response and plasma amphetamine level at 2 and 4 hours post administration. Studies of psycholinguistic parameters with Dr. Ludlow (NINCDS) show that hyperactive children treated with amphetamine compared to placebo show an increase in language comprehension, speak more slowly, produce fewer echolalic and stereotypic responses, and produce relatively less speech not relevant to examiner requests. These projects are discussed in greater detail in Projects Z01 MH 00100-02 AP and Z01 MH 00040-02 AP of the Biological Psychiatry Branch.

Significance to Biomedical Research:

Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods for determining central nervous amine utilization in man is essential for testing hypotheses regarding the role of amines in psychiatric, medical, and neurological disorders and assessing the effects of drugs on the amines in the brain. Some of the metabolic strategies under development will have broader applications. For example, the stable isotope technique for determining the turnover rate of methionine may be a useful tool to study some disorders of amino acid metabolism, as well as brain catecholamine metabolism.

Proposed Course:

The techniques described above are now being applied to studies in patients. When appropriate, some of the above techniques will also be studied next year in normal volunteers in order to generate a comparison group to patients with various disorders of neurotransmission.

Publications:

1. Ziegler, M.G., Lake, C.R., Wood, J.H. and Ebert, M.H.: Circadian rhythm in cerebrospinal fluid noradrenaline of man and monkey. Nature 264 (5587): 656-658, 1976.

2. Perlow, M.J., Gordon, E.K., Ebert, M.E., Hoffman, H.J. and Chase, T.N.: The circadian variation in dopamine metabolism in the subhuman primate. J. Neurochem. 28: 1381-1383, 1977.

3. Marynick, S.P., Havens, W.W., Ebert, M.H. and Loriaux, D.L.: Studies on the transfer of steriod hormones across the blood-cerebrospinal fluid barrier in the rhesus monkey. Endocrinology 99 (2): 400-405, 1976.

4. Ziegler, M.G., Lake, C.R., Wood, J.H., Brooks, B.R. and Ebert, M.H.: Relationship between norepinephrine in blood and cerebrospinal fluid in the presence of a blood-cerebrospinal fluid barrier for norepinephrine. J. Neurochem. 28: 677-679, 1977.

5. Perlow, M.J., Festoff, B., Gordon, E.K., Ebert, M.H., Johnson, D.K. and Chase, T.N.: Daily fluctuation in the concentration of cAMP in the conscious primate brain. Brain Res. 126: 391-396, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00352-02 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	E. D. Caine	Clinical Associate	LCS NIMH
	H. Weingartner	Guest Worker	BPB NIMH
	R. Kartzinel	Guest Worker	LCS NIMH
	C. Ludlow	Speech Pathologist	CDP NINCDS

COOPERATING UNITS (if any)

Communicative Disorders Program, NINCDS
Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Experimental Therapeutics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several neuropsychiatric syndromes including Huntington's chorea, Altzheimer's disease, and Gilles de la Tourette's syndrome are being studied in terms of memory and intellectual function, neuroendocrine function, and possible pharmacological treatment. Huntington's patients are unable to utilize normally significant aids such as high verbal imagery to encode information deeply enough to allow consistent retrieval. A study of dopaminergic regulation of prolactin and growth hormone in Huntington's patients and normal controls indicates that there are deteriorated dopamine mediated control mechanisms for prolactin release in Huntington's chorea. Acute pharmacological testing with d-amphetamine, l-amphetamine, and haloperidol in Gilles de la Tourette's syndrome suggests that the pathophysiology may be biochemically heterogeneous. Clozapine, a non-neuroleptic antipsychotic is being studied in Tourette's patients. Clinical trials of two psychotropic drugs known to stimulate appetite (lithium carbonate and Δ-9-tetrahydrocannabinol) are underway in anorexia nervosa. Abnormalities in gastric function and cardiac rhythm have been documented in acute anorexia nervosa.

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neuroendocrine function, neurotransmitter metabolism, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Huntington's dementia and Altzheimer's disease. Other diseases under intensive study are Gilles de la Tourette's syndrome and anorexia nervosa.

Methods Employed: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short term memory; (2) long term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these processes and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory. These methods have previously been validated and studied in a number of clinical and psychopharmacological studies. The procedures to measure short term memory functions include immediate recall of random high and low imagery words, consonant trigrams, taxonomically organized words, digits, and forms generated from mutually exclusive binary dimensions. Long term memory functions are tested by using equivalent sets of stimuli, with varying periods of delay. Rehersal is prevented through the insertion of appropriate, noninterfering tasks. Differences between free recall and various cued recall conditions is examined. Encoding strategies associated with normal versus disturbed brain states is measured using sorting tasks having redundant and random word stimuli.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of various etiologies, focusing on the adrenergic and cholinergic systems. A similar study of drugs that stimulate or block dopaminergic neurotransmission has been completed in Gilles de la Tourette's syndrome.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses one can infer the functional state of dopaminergic transmission in the tuberoinfundibular system. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double blind design, appropriate rating scale placebo controls. Methods used in studies of neurotransmitter metabolism are those methods outlined in Project Z01 MH 00351-02 LCS.

Major Findings: The adult neuropsychiatric program continued to focus on the dementia of Huntington's disease [Dr. Caine, Dr. Weingartner (BPB)]. Affected individuals are unable to utilize normally significant

aids (e.g., high verbal imagery) for encoding information deeply enough to allow consistent retrieval. Patients fail also to process new items at a normal rate of speed. Psychophysiological studies have begun in collaboration with Dr. Evarts (LNP) and Dr. Ludlow (NINCDS) to investigate the ability of Huntington's patients to utilize kinesthetic and auditory stimuli. A pilot study of pharmacological effects on memory function is in progress. The effect of acute cholinergic and dopaminergic stimulation and blockade on various parameters of memory function in Huntington's patients and normal controls is being studied.

The study of Gilles de la Tourette's syndrome (Dr. Caine) has enlarged its scope, having evaluated 30 patients in the last year and admitted 11 for intensive study. Acute, provocative, pharmacological testing with d-amphetamine, l-amphetamine and haloperidol suggests that the pathophysiology may be biochemically heterogeneous. Linguistic studies (with Dr. Ludlow) demonstrate language and speech deficits in most patients; the former is highly correlated with an increased variety of vocalizations. The problem of tardive dyskinesia in Tourette's patients has become a focus for developing new therapeutic agents. Clozapine, a non-neuroleptic antipsychotic, is being studied intensively in Tourette's patients. Drug trials using the same agent have begun in tardive syskinesia and Huntington's disease. This agent may have considerable clinical significance because it has not been reported to cause tardive dyskinesia.

A neuroendocrine protocol completed this year demonstrated abnormalities in prolactin regulation in Huntington's chorea. Baseline plasma prolactin levels are abnormally high as compared to healthy controls; further patients fail to respond to the suppressive effects of l-dopa and bromocriptine. Although they have increased prolactin release after chlorpromazine, their maximum prolactin levels are significantly lower than controls. These results suggest that Huntington's patients have deteriorated dopamine mediated control mechanisms for prolactin release.

A clinical research program in anorexia nervosa has been fully developed this year by Dr. Gross. Patients who are acutely ill are being admitted for study in a program which includes a behavior modification regimen as a baseline treatment. An initial evaluation period includes several studies of central and peripheral monoamine metabolism. Several drug trials of agents known to stimulate appetite are underway. Preliminary results of a trial of lithium carbonate in promoting weight gain in acute anorexia nervosa suggest that it is efficacious. A clinical trial of Δ-9-tetrahydrocannabinol has been approved by the FDA and will begin soon. A study of gastric function in anorexia nervosa with Dr. Dubois (National Naval Medical Center) has demonstrated that during the acute phase, fractional emptying rate is decreased twofold, and intragastric volume is increased twofold. A study of cardiac function during the acute phase with Dr. Gottdiener, Henry, and Borer (NHLBI) indicates that although left ventricular mechanical function is intact, ventricular extrasystoles (VAC's) occur frequently and in clear association with decreased left

ventricular diastolic dimension and wall thickness. Data collection on psychological characteristics that will predict favorable response to behavior modification or drug treatment is in progress.

Significance to Biomedical Research:

Despite a European tradition of neuropsychological research throughout this century, it has only been in the past 15-20 years that interest in cognitive functioning has increased significantly in thia country, as seen for example in the works of Talland, Goodglass, or Sperry. The biological mechanisms which underlie memory processing remain unclear. In recent years, interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. However, there is a beginning literature of clinical neuropharmacological investigations of memory, including work on stages of memory storage pursued recently in our program by Drs. Weingartner and Murphy. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology.

Gilles de la Tourette's syndrome is a neuropsychiatric syndrome whose pathophysiology is poorly understood. The symptomatology appears to be responsive to drugs which block dopamine receptors. By conducting new drug trials, and gathering data on cognitive and linguistic function, monoamine metabolism, and neuroendocrine function in this illness, we hope to expand knowledge about its pathophysiology and its possible relationship to other psychiatric illnesses such as schizophrenia. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite.

Proposed Course:

The course of this project is related to the growth and development of clinical research unit 2 West as a central nervous system clinical pharmacology service. New diseases may come under study as indicated by available laboratory techniques in the LCS. In the next year we intend to further pursue pharmacological studies of memory function in Huntington's chorea, Altzheimer's disease, and normal controls. We also plan to gather more data on the utilization of auditory and kinesthetic stimuli by Huntington's patients. In Gilles de la Tourette's syndrome we intend to complete a clinical trial of clozapine, initiate new drug trials, and begin systematic studies of monoamine metabolism in these patients. In anorexia nervosa, we intend to complete drug trials of lithium carbonate and Δ-9-tetrahydrocannabinol, extend studies of monoamine metabolism that have

begun this year, and gather psychometric data on those psychological characteristics that predict favorable response to behavior modification or drug treatment.

Publications:

1. Caine, E.D., Weingartner, H., and Ebert, M.H.: An outline for the analysis of dementia: The memory disorder of Huntington's disease. Neurology, in press.

2. Caine, E.D., Hunt, R., Weingartner, H. and Ebert, M.H.: Huntington's dementia: Clinical and neuropsychological features. Arch. Gen. Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00271-08 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Assay of Amine Metabolites in Human Biological Fluids to Estimate Rates of Amine Metabolism in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. K. Gordon	Chemist	LCS	NIMH
Other:	I. J. Kopin	Chief, LCS	LCS	NIMH
	J. A. Oliver	Chemist	LCS	NIMH
	R. L. Sherman	Biologist	LCS	NIMH
	P. Blombery	Guest Worker	LCS	NIMH
	S. P. Markey	Pharmacologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gas liquid chromatography- mass spectrometry (GLC-MS), ion exchange chromatography, radioisotopic techniques and many separation procedures are used to develop assays and measure biogenic amines and their metabolites in cerebrospinal fluid, blood and urine. Appropriately labelled deuterium tritium or carbon 14 compounds are synthesized or prepared enzymatically for use as internal standards or for studies in patients with mental and neurologic disorders.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Specific and sensitive assays for metabolites of biogenic amines in cerebrospinal fluid, blood, and urine are required for clinical studies of amine metabolism in human subjects. Many such studies involve use of heavy or radioactive isotopes and accurate measurement of specific activities is essential for kinetic analyses of the results. The role of the Unit on Clinical Biochemistry is in the development and use of assay procedures to meet these requirements.

Methods for separation and assay by liquid and gas chromatography of a major metabolite of norepinephrine in brain, 3-methoxy-4-hydroxyphenylglycol (MHPG) and its glucuronide and sulfate conjugates in CSF, plasma, and urine is being applied to determination of the specific activities of these metabolites after infusion of ^{14}C labelled dopamine.

Studies on the effects of several drugs on amine metabolites in CSF (Mrs. Gordon) are being carried out in collaboration with investigation in other divisions, e.g., Effects of haloperidol on rat striatal dopamine synthesis (Dr. P. Lermer, NHLI), of lergotril or GABA on dopamine metabolism in parkinsonian patients (Drs. Calne and Teychenne, NINCDS), and of probenecid (Drs. Ebert and Goodwin, NIMH) on acidic metabolites in CSF. Conjugates of 3,4-dihydroxyphenylacetic acid (DOPAC) have been found in human as well as monkey CSF (Mrs. Gordon, Dr. Markey, and Mr. Sherman). The role of the spinal cord in contributing and removing amine metabolites in lumbar spinal fluid has been assessed (Dr. Kessler, Mrs. Gordon, and Dr. Reid) and circadian rhythms in amine metabolites in ventricular CSF of monkeys demonstrated (Dr. Perlow, Mrs. Gordon, Drs. Ebert, Hoffman, and Chase). Use of mass spectroscopy to measure levels of incorporation of ^{18}O into HVA (Mr. Oliver, Dr. Ebert, and Mrs. Gordon) have revealed that the decline in ^{18}O in ventricular ^{18}O-labelled HVA reflects turnover of the ^{18}O-labeled dopamine. This method, combined with use of CD_3-labeled methionine, may be useful to determining rates of amine turnover in human brain.

PUBLICATIONS:

Gordon, E.K., Markey, S.P., Sherman, R., and Kopin, I.J.: Conjugated 3,4-dihydroxyphenylacetic acid (DOPAC) in human and monkey cerebrospinal fluid and rat brain and the effects of probenecid treatment. Life Sci. 18: 1285-1292, 1976.

Kessler, J.A., Gordon, E.K., Reid, J.L., and Kopin, I.J.: Homovanillic acid and 3-methoxy-4-hydroxyphenylethyleneglycol production by monkey spinal cord. J. Neurochem. 26: 1057-1061, 1976.

Perlow, M.J., Festoff, B., Gordon, E.K., Ebert, M.H., Johnson, D.K. and Chase, T.N.: Daily fluctuations in the concentration of cAMP in the conscious primate brain. Brain Res. 126: 391-396, 1977.

Perlow, M.J., Gordon, E.K., Ebert, M.H., Hoffman, H.J., and Chase, T.N.: The circadian variation in dopamine metabolism in the subhuman primate. J. Neurochem. 28: 1381-1383, 1977.

Lerner, P., Nose, P., Gordon, E.K., and Lovenberg, W.: Haloperidol: Effect of chronic treatment on rat striatal dopamine synthesis and turnover. Science 197: 181-183, 1977.

Wyatt, R.J., Cantor, F., Gillin, J.C., Gordon, E.K., Karoum, F., McCullough, D., Neff, N., Ommaya, A., Ransaker, F.P., Seaberg, J.B., and Slaby, A.: Ventricular fluid metabolites of phenolic amines and catecholamines. In Usdin, E. and Sandler, M. (Eds.): Trace Amines and the Brain, Psychopharmacology Series. New York, Marcel Dekker Inc., 1976, vol. 1, pp. 209-232.

Kopin, I.J.: Measuring turnover of neurotransmitters in human brain. The College of Neuropsychopharmacology, New Orleans, 1977, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00272-06 LCS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism, Distribution and Biochemical Effects of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. P. Markey	Pharmacologist	LCS NIMH
OTHER:	R. L. Colburn	Chemist	LCS NIMH
	A. P. Zavadil	Research Associate	LCS NIMH
	A. J. Lewy	Staff Psychiatrist	CP NIMH
	R. L. Hawks	Guest Worker	LCS NIMH
	J. Johnson	Guest Worker	FDA
	G. J. Shaw	Visiting Fellow	CH NHLBI
	A. W. Hoveling	Guest Worker	CH NHLBI
	I. J. Kopin	Chief, LCS	LCS NIMH
	M. H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	D. B. Calne	Clinical Director, NINCDS	NINCDS NIH

COOPERATING UNITS (if any)

Sections on Medicine and Experimental Therapeutics, LCS; Clinical Psychobiology Branch, NIMH; Cardial Renal Division, FDA; Laboratory of Chemistry, NHLBI; Intramural Research Program, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the metabolic fate of drugs and their biochemical effects in animals and man are being pursued in order to relate pharmacological action to molecular events and clinical efficacy. Work in progress includes determination of the mode of action of bromocryptine in animals, synthesis and chronic testing of 4-phenyl-4-propionoxy-N-methylpiperidine and related compounds in animals, imipramine and desipramine levels in various patient groups, and the pharmacokinetics of cocaine in humans.

PROJECT DESCRIPTION:

Bromocryptine, a dopamine agonist effective in the treatment of Parkinson's disease has been studied in rats and primates. The levels of unchanged radiolabeled (^{82}Br) drug reaching the central nervous system are less than 10^{-8} molar, and an effort to prepare radiochemically pure bromocryptine has been undertaken.

The analgesic 4-phenyl-4-propionoxy-N-methylpiperidine and several analogs have been synthesized and are being chronically administered to animals. The intent is to determine whether their long term effects mimic the apparent Parkinson-like syndrome developed by a drug abuser exposed to these compounds. Studies of the dopamine-rich brain regions will be undertaken using gas chromatographic-mass spectrometric methods.

Imipramine and desipramine levels have been studied in blood, CSF, and saliva in patients with primary affective disorders. Pharmacokinetic studies of acute and chronic losses of imipramine in humans are in progress.

Continued synthesis of labeled internal standards for mass spectral studies has resulted in an improved general procedure for production of highly deuterated diazomethane. Deuteromethylated homovanillic acid and tritiated 4-hydroxy-3-methoxyphenylglycol have also been prepared.

Studies on the human pharmacokinetics of cocaine are in progress using a gas chromatographic-mass spectrometric method.

PUBLICATIONS:

Claeys, M., Markey, S.P., and Maenhaut, W.: Variance analysis of error in selected ion monitoring assays using various internal standards. A practical study case. Biomed. Mass Spectrom. 4: 122-128, 1977.

Claeys, M., Muscettola, G., Goodwin, F.K., and Markey, S.P.: Measurement of imipramine and desipramine in plasma, cerebrospinal fluid and saliva of patients with primary affective disorders. Proc. Int. Symp. on Quantitative Mass Spectrometry in the Life Sciences, Ghent, Belgium, 1976.

Shaw, G.J. and Markey, S.P.: Synthesis of N-methyl-^{2}H-desipramine and imipramine: Non-exchangeable standards for pharmacokinetic studies by mass spectrometry. J. Labelled Compd. (In press.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00273-08 LCS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Synaptosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. W. Colburn	Chemist	LCS NIMH
OTHER:	N. B. Thoa	Pharmacologist	LCS NIMH
	I. J. Kopin	Chief, LCS	LCS NIMH
	I. C. Campbell	Visiting Fellow	CN NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synaptosomes prepared from various regions of rat brain are used as models of nerve endings to study mechanisms of uptake and release of neurotransmitters in the central nervous system. The distribution of neurotransmitters within synaptosomes are determined by hypotonic shock on millipore filters following neurotransmitter uptake or uptake plus drug-induced release. Depolarizing levels of K+ releases norepinephrine and serotonin from both the shock resistant and shock susceptible fraction.

PHS-6040

PROJECT DESCRIPTION:

Synaptosomes prepared from various regions of rat brain are used as models of nerve endings to study mechanisms of uptake and release of neurotransmitters in the central nervous system. When neurotransmitter uptake is followed over time with hypotonic shock treatment on millipore filters there is a preferential uptake in the shock resistant fraction during early (1-2 min.) time periods. If the accumulated transmitter is released by depolarizing K+ levels there is a calcium-dependent release from the shock resistant fraction and a calcium-independent release from the shock susceptible fraction.

Studies with I. C. Campbell show that monoamine oxidase in synaptosomes is inhibited by clorgyline a type A inhibitor but not by deprenyl a type B inhibitor.

Additional studies with synaptosome preparations were with tri-iodothyroxine (with M. B. Dratman) and with morphine (with A. K. Ho).

PUBLICATIONS:

Rajan, K.S., Colburn, R.W., Davis, J.M.: Metal Chelates in the Storage and Transport of Neurotransmitters. In Sigel, H. (Ed.): Metal Ions in Biological Systems, Vol. 6. New York, Marcel Dekker Inc., 1976, pp. 291-321.

Dratman, M.B., Crutchfield, F.L., Axelrod, J., Colburn, R.W. and Thoa, N.B.: Localization of tri-iodothyronine in nerve ending fractions of rat brain. Proc. Natl. Acad. Sci. 73: 941-945, 1976.

Ho, A.K., Ng, L.K., Thoa, N.B., and Colburn, R.W.: In Vitro Interactions on Synaptosomal Uptake of Morphine and Biogenic Amines. In Ford, D. and Clouet, D. (Eds.): Tissue Responses to Addictive Drugs. New York, Spectrum, 1976, pp. 209-218.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00274-03 LCS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Methods of Ionization in Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. P. Markey	Pharmacologist	LCS NIMH
OTHER:	A. J. Lewy	Staff Psychiatrist	CP NIMH
	A. W. Hoveling	Guest Worker	CH NHLBI

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, NIMH
Laboratory of Chemistry, NHLBI
Section on Technical Development, NIMH
Computer Systems Laboratory, DCRT

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Enhanced sensitivity in mass spectrometry can be achieved by increased efficiency of ionization. An instrument has been modified for negative chemical ionization mass spectrometry which detects electron capturing molecules with 10-1000-fold improved sensitivity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

A quadrupole mass filter was modified (a) by construction of a flange permitting the inclusion of two electron multipliers, one with a high voltage co-axial feed thru for negative ion output and (b) with a new configuration of an electron multiplier to detect negative ions. After the former mode was found to perform unstably, the latter was tested and found to exhibit high grain with very low noise. Negative ions are attracted to the first dynode of a 16-stage multiplier by +2kv. The second dynode is kept at -2.5kv and the output signal is referenced to ground as in a conventional electron multiplier. Conversion of negative ions to positive ions apparently occurs on the surface of the first dynode.

A number of biogenic amine metabolites have been analyzed as their perfluoroacyl esters and were found to exhibit lower levels of detectability than with other modes of ionization. An assay capable of measuring plasma levels of melatonin is being developed.

PUBLICATIONS:

Markey, S.P., Lewy, A.J., Zavadil, A.P., Poppiti, J.A., and Hoveling, A.P.: Quantitation of biogenic amines and metabolites using negative ion chemical ionization. Proc. 25th Ann. Conf. on Mass Spectrometry and Allied Topics, Washington, D.C., 1977. (In press.)

Tate, R.L., Barden, L.K., Schultz, A.R., and Markey, S.P.: Selected ion recording minicomputer data system for magnetic deflection mass spectrometers. Proc. 25th Ann. Conf. on Mass Spectrometry and Allied Topics, Washington, D.C., 1977. (In press.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00221-17 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Behavior in the Preschool Child: Cross-Sectional Analyses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.F. Halverson, Jr.	Research Psychologist	LDP NIMH
OTHER:	G.E. Inoff	Psychologist	LDP NIMH
	M.F. Waldrop	Guest Worker	LDP NIMH

COOPERATING UNITS (if any)
None

LAB & BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.05	.50	1.55

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the organization of social and cognitive behaviors in 3-year-old children. Samples consist of 65 males and 66 females. Data from a research nursery school are based on observations in three different free play settings as well as performance in a number of experimental tests of coping with barrier situations. Reliable subjective ratings are included to cover the areas of social, emotional and attentional behavior. Cognitive and language behaviors in the young children are assessed by a variety of standardized tests. Analyses cover sex-differences, activity level, and cross-setting and cross-time stabilities of behavior.

Project Description:

This research is directed to the organization of social and cognitive behaviors in three-year-old children. (1) What are the stabilities over time and across situations of the child's social and play behavior? Specifically, how situationally and age dependent are the young child's temperamental behaviors? What situational influences (e.g., number of toys, age, and sex composition of group) affect the expression of positive and negative social interaction? How are the child's behaviors observed in the nursery school related to family and social experiences at home? (2) What characteristics of the child (e.g., activity level, cognitive-intellectual maturity, sex of child, attention-span) influence social behavior towards peers and adults? How reliable are individual and sex differences in activity level and how important are they in a child's social and cognitive behavior? How is the child's relationships with adults (mother, teachers) related to peer interaction? (3) How are the cognitive and social behaviors of the child related to coping with frustrating situations?

Subjects are 131 children, ages two years four months to three years six months, from middle class white families. Children attended a 4-week nursery school session in mixed-sex groups of 5 children per group. Interest has been focussed on the child's attentional deployment, social behavior towards peers and caretakers, and the nature of assertive and coping behaviors. Measures were based on direct naturalistic observation and teacher ratings in settings of indoor and outdoor play, rest, and refreshment. Activity recorders worn by the children provided measures of activity level. Experimental procedures were designed to elicit the child's coping and assertive behaviors when confronted with physical barriers or novel situations. Standardized tests of verbal and cognitive development were administered. Data were obtained from the mothers in detailed diary accounts covering three weeks of family life, and in an interview focussing on attitudes toward aggression, behavioral control and child rearing in general.

The project was designed with many variables similar in content, but coming from different procedures. The interrelations of various behavioral dimensions and their contextual determinants will be examined. For some purposes the analysis strategy has been to concentrate on forming clusters of variables that have cross-setting and cross-method generality and stability.

Analyses of sex differences in high activity behaviors indicate that although high-active, intense play and social behavior is a major feature of both boys' and girls' behavior at age three, important differences exist between the sexes. For boys, high magnitude behavior is characterized by "intrusive" behaviors, while high magnitude behavior for girls is represented by high levels of positive social interaction. The effects of high-activity behavior on parents are different for boys and girls. For boys, high-activity behaviors elicit negative evaluations from parents, while high-activity behaviors on the part of girls elicit positive evaluations from parents. This sex difference is maintained even when the two sexes are equated for activity level. Active boys appear to be difficult and hard to control and active girls to be social

and outgoing.

One analysis has been published this year. Gale Inoff and Charles Halverson assessed relations between observed behaviors in a particular circumscribed caretaker-child interaction setting and child behavioral dispositions which were assessed by more extensive observation. Caretaker-child interaction data were collected across several days in the nursery school free-play setting and were related to child behavioral disposition data which were collected across 4 weeks of nursery school attendance. For children of both sexes, child behavioral disposition variables which described out-of-control and disruptive behaviors were positively related to level of caretaker-initiated interaction in the caretaker-child interaction situation. For boys, child behavioral disposition variables describing impatience, excitement, and outgoingness and, for girls, child behavioral disposition variables describing nonaggressiveness and fear were positively related to level of child-initiated interaction in the caretaker-child interaction situation. The caretaker's initiations were interpreted as responses to what are traditionally viewed by adults as "problem" behaviors. The child's initiations were interpreted as reflecting the child's own need for comfort and nurturance. There was a sex difference in the bases of such a need, namely, high intensity activities for males and low intensity activities for females.

A final report on activity level in the 3-year-old sample is in preparation and will be submitted for publication in the coming year. This is the final report on this project.

Publications:

Inoff, G.E. and Halverson, C.F., Jr: Behavioral disposition of Child and Caretaker-Child Interaction. Dev. Psychol. 13, No. 3: 274-281, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00223-03 LDP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Relation of Problem Behaviors and Minor Physical Anomalies in Young Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.F. Waldrop	Guest Worker	LDP NIMH
OTHER:	C.F. Halverson, Jr.	Research Psychologist	LDP NIMH
	R.Q. Bell	Prof. Dept. of Psychology	Univ. of Va., Charlottsville, Va.
	R.K. Yang	Assist. Prof. Dept. of Child & Family Dev.	Univ. of Georgia Athens, Ga.

COOPERATING UNITS (if any)

None

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.40	1.00	.40

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, behavior of males at age 3 was predicted from a 5 to 10 minute newborn examination. The number of minor physical anomalies, assessed soon after birth, was significantly related to short attention span, peer aggression, impulsivity and nomadic activity at age 3. Children scoring high on these behaviors are frequently labeled "hyperactive." Standardized anomaly scores were plotted against factor scores representing measures of hyperactivity. Very few of the boys who had scores higher than the mean on anomalies had scores lower than the mean on factors describing short attention span, peer aggression, and impulsivity. There were very few false positives, and none of these was extreme. For girls, newborn anomaly scores were associated with withdrawal, low peer involvement, low verbal expressiveness, and poor enunciation at age 3.

Project Description:

The purpose of this investigation was to assess the relations between minor congenital physical anomalies and later problem behaviors.

The sample was a cohort of 118 three-year-old children (59 boys and 59 girls) forty-eight (23 boys and 25 girls) of whom were seen as newborns. At the newborn period and at the preschool period, each child was examined for the presence of 16 or 17 minor physical anomalies, such as head circumference out of normal range, epicanthus, hypertelorism, low set ears, high steepled palate, curved 5th finger, single transverse palmar crease, and 3rd toe longer than 2nd. The minor physical anomalies are presumed to be the result of developmental deviations resulting from either some form of genetic transmission or from insults in early pregnancy which mimic genetic transmissions. The score for anomalies was the cumulative number of anomalies present plus, for some anomalies, extra eight when the anomaly was judged to be extreme.

At age 3, measures of hyperactive behavior were assessed during attendance at a nursery school. The word "hyperactive" refers to the upper range of a behavioral dimension that is normally distributed in the general population of young children. The cluster of behaviors making up this dimension, however, does include the same behaviors that are included in what is known as the "hyperactivity syndrome" in the literature referring to children in clinical treatment programs.

The nursery school measures were factor scores based on reliable observations and teacher ratings and were assessed independently of the anomaly index. The nursery school measures were: Short attention span and Peer aggression and impulsivity.

Both newborn anomalies and anomalies assessed at age 3 significantly correlated with the nursery school factor scores for boys: Short attention span with newborn anomalies (r = .57, p <.001) and with anomalies at age 3 (r = .35, p <.01); Peer aggression and impulsivity with newborn anomalies (r = .51, p <.01) and with anomalies at age 3 (r = .32, p <.01).

To obtain one score for hyperactivity, the two factors, Short attention span and Peer aggression and impulsivity were summed. The resulting scores correlated .67 (p <.01) with the newborn anomaly score. Thus, close to half the variance of the nursery school measure of hyperactivity was accounted for by its relation to newborn minor physical anomalies.

There were very few boys with higher than the mean anomaly score who had scores lower than the mean on either factor. There were very few false positives and none of these was extreme. There were, however, more false negatives. This indicated that it was not unusual for boys who were seen as hyperactive to have had low anomaly scores, but it was very unusual for boys with high anomaly scores not to be seen as hyperactive at age 3.

An important implication of this study is that an early anomaly assessment makes probable the early detection of some boys who are likely to exhibit hyperactive behaviors at age 3. Since hyperactivity at the preschool age has been found to be related to hyperactivity at age 7, it would be reasonable to expect the newborn anomaly assessment to predict hyperactivity for boys at the elementary school age. This is yet to be done. The results of this study indicate there is a congenital contributor to some frequently occurring behavior problems in the general population of young boys.

The results for girls are less clear. The newborn anomaly score for girls is associated with withdrawal, being cautious, not being involved with peers, low verbal expressiveness and poor enunciation at age 3.

Studies in the relation of problem behaviors to the frequency of minor physical anomalies have aided in understanding the interplay between possible constitutional factors and developing cognitive, social and emotional behaviors in young children. The findings of these studies do not indicate constitutional predetermination of early behavioral problems. A more likely hypothesis is that it is the interaction of congenital and experiential factors that lead to the expression of difficult behaviors in early childhood.

A paper reporting the findings from the longitudional sample (neonate to age 3 for males) has been submitted for publication. This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00224-03 LDP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Data from Preparental, Newborn, Mother-Infant, and Preschool Studies from the Longitudinal Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.F. Halverson, Jr.	Research Psychologist	LDP NIMH
	H.A. Moss	Research Psychologist	LDP NIMH
OTHER:	M.F. Waldrop	Guest Worker	LDP NIMH
	R.K. Yang	Assist. Prof. Dept. of Child & Family Dev.	Univ. of Georgia

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Developmental Psychology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.90	.40	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Longitudinal data were collected at four phases of family development: (1) preparental; (2) neonatal; (3) mother-infant; and (4) preschool. Three analyses are reported. The first involves continuity between neonatal and 3-month infant behavior. Few significant associations were found. There is some continuity for males, but not for females. The second analysis concerns endogenous and exogenous contributors to vocal behavior. Findings suggest that males exhibit endogenous continuity, while females are more responsive to environmental (exogenous) influences. Antecedents of preschool activity, examined in the third analysis, were found for males, suggestive of endogenous continuity.

Project Description:

The purpose of this research is to interrelate data from the following developmental stages, (1) preparental, (2) neonatal, (3) mother-infant, and (4) preschool, to determine the joint contribution of congenital and environmental influences on development.

Data have been collected by four separate teams of investigators on each of the developmental phases under study. Procedures are described in earlier Annual Reports. Reduction of the initial sets of measures into manageable and optimally functional sets of variables has been accomplished. Details of the data reduction procedures have also been described in earlier reports.

Three cross-stage analyses have been carried out:

Study A: Relations between neonatal and 3-month behavior (Yang and Moss)

At the newborn period, assessments were made of sleeping patterns and sucking and crying responses to stimulation during waking periods. At three months naturalistic home observations of mother and infant provided data on infant characteristics. Factor analyses within periods and correlation analyses between periods yielded relations differing for the sexes. Few significant relations were found. For males, newborn level of maturity was positively correlated with tonic-active behavior at three months ($r = .37$, $p < .02$). For females, newborn reactivity-irritability was positively correlated with clarity of infant behavior at three months ($r = .30$, $p < .10$). Correlations between the components of the male factors supported the factor relationship For females, however, correlations between the components did not support th relationship between the factors. Males who at 3 months were visually alert strong, and active, were as newborns characterized by long gestational age, large body size, and low autonomic variability during sleep (within clinical normal boundaries). This analysis has been submitted for publication.

Study B: Analysis of endogenous and exogenous contributors to vocal behavior (Moss, Halverson, Yang)

Variables associated with infant vocal behavior were studied using data from pregnancy interviews, newborn observations, three-month home observations, an infant vocal conditioning procedure and a maternal interview at 3½ month and nursery school observations of vocal behavior at three years.

Findings emerging from these analyses indicated social and endogenous factor to be associated with vocal behavior, but that social factors tended to be stronger for females while endogenous factors were stronger for males. For example, a rating of the pregnant mother's preference for an active, high-drive infant was correlated for males with newborn spontaneous crying ($r = .41$, $p < .01$), three-month infant vocalizations ($r = .27$, $p < .05$) in the home, and vocalization in the conditioning procedure ($r = .25$, $p < .10$). Newborn arousal (spontaneous crying and head lifting) correlated with vocalizing ($r = .49$, $p < .01$, $r = .32$, $p < .05$), and "kicking and thrashing"

(r = .38, p < .01, head lifting only) in the home observation, and vocalization (r = .38, p < .01, head lifting only) during the conditioning procedure. Also, for males, vocalization in the home was predictive of vocalizing in the conditioning procedure (r = .44, p < .001) and in the nursery school (average r = .31, p < .05) three years later. In addition, a rating of the mother as "animated" and having a stimulating quality to her voice was related to her preference for an active infant (r = .48, p < .001).

For females a measure of contingent maternal vocalization in the home and a high rating on interest in social interaction with infants was more strongly related to vocalization during conditioning (r = .29, p < .05, r = .40, p < .05) and vocabulary score at nursery school (r = .44, p < .001, r = .23, p < .10). In addition, interest in social interaction with infants during the first three months of life was significantly correlated with the female's verbal behavior at three years of age.

Study C: Relations between infant characteristics and preschool activity level (Halverson, Moss, Yang, Waldrop)

This study related factors derived from neonatal measures and from observations of infant behavior at 3 months with a large cluster summarizing both social and physical activity (to be referred to as "activity level") at three years of age.

Results indicate that activity level at three years of age has clear origins in early infancy for males, but not for females. For males, neonatal maturity and arousal were negatively correlated with activity level at age three years (r = -.28, p < .05; r = -.37, p < .01). Also, tonic-active behavior (visual alertness, strength, and activity) as well as social behavior at three months of age were correlated with activity level at nursery school (r = -.33, p < .02; r = .47, p < .001).

These relations describe two developmental lines relating to highly active preschool behavior. One line can be characterized as an "inversion of intensity" between the periods. As described by the first 3 correlations presented above, low magnitude behavior at newborn and three months of age predict high magnitude behavior at age three years. The second developmental line, as described by the fourth correlation presented above, is a direct isomorphic link between social behaviors at three months of age and activity level at three years: High levels of early social behavior predict later high levels of activity.

For girls, however, the early data generally do not predict later activity level. Of the four newborn factors, only arousal is (negatively) related to activity level (r = -.30, p < .05), supporting the "inversion of intensity" discussed for boys. No behaviors at three months of age predicted later activity level.

Girls' activity level seems to have few common antecedents with boys' activity level. It seems possible that high activity in boys has a more congenital basis and those same behaviors in girls may have a more social or environmental

origin.

Final reports of the latter two studies are in preparation and will be submitted for publication in the coming year. This is the final report of this project.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00228-10 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Determinants and Dimensions of Mother-Infant Interaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.A. Moss	Research Psychologist	LDP NIMH
OTHER:	B.S. Jacobs	Psychologist	LDP NIMH
	S.J. Kearns	Psychologist	Formerly with CRB NIMH

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.65	.30	.35

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A sample of 121 mother-infant pairs were studied in order to identify the primary dimensions of mother-infant interaction and to relate these data to other information obtained in a longitudinal program on family development. Data consist of interviews and questionnaires with expectant mothers during the third trimester of pregnancy; time-sampled codings from two 6-hour home observations when the infants were three months of age; postpartum interviews and questionnaires administered to the mothers; a series of laboratory procedures conducted in the homes to assess stranger and separation reactions, imitation, socialization, and exploratory behavior when the infants were about eleven months of age. Relations were found between maternal attitudes and maternal behavior and these variables interacted with the socioeconomic status of the mother and with the sex of the infant. Results have also been obtained demonstrating a positive correlation between the frequency of contingent maternal behavior toward infant vocalizations in the home and the rate at which these infants vocalize to similar contingent reinforcements in a laboratory procedure.

Project Description:

The major objective of this research is to identify primary dimensions of mother-infant interaction.

The sample consists of 121 mother-infant pairs of lower-middle to upper-middle class background.

The mother-infant studies consist of: (1) Interviews and questionnaires administered to the expectant mothers during the last trimester of pregnancy, concerning attitudes and expectations pertaining to pregnancy and motherhood. (2) Two six-hour home observations of mother-infant interaction at three months of age. Maternal variables include caretaking activities, contingent responsiveness, and social and affectionate behaviors toward the infant. Infant variables emphasize state characteristics and social behaviors. (3) Postpartum interviews and questionnaires administered to the mother when the infant was about 3½ months of age, on dimensions similar to those studied from the pregnancy material. This allows evaluation of the continuity of maternal attitudes and the effect of infant characteristics on the stability of these maternal attitudes. (4) Infant vocal conditioning, visual attention, resistance to cuddling, and muscle tonus, studied in the laboratory. (5) Home observations when the child was eleven months of age, involving reation to strangers, separation from the mother, imitation, socialization and exploratory behavior.

A principal component factor analysis was computed for each set of data in order to simplify the data base and identify the primary dimensions underlying each major procedure. Summary descriptions of these factors are reported in an earlier annual report of this project (Z01 MH 00228-08 LDP). Factor scores were derived for each of these factors and these scores were correlated with one another across procedures. This permitted evaluation of the stability of maternal attitudes from pregnancy through 3½ months of age as well as assessment of the degree to which these attitudes were related to maternal and infant behavior. This analysis was conducted separately for males and females and for the upper half and lower half of the socio-economic distributions of the sample. There was moderate stability for functionally similar attitude factors from the pregnancy to the postpartum phase and for the upper half of the SES distribution. Those correlations that did occur generally replicated for subsamples of males and females. For instance, the pregnancy factor dealing with a "positive" orientation toward infants and the maternal role was significantly correlated with the frequency with which the higher SES mothers kissed their infants during the 3-month home observations ($r = .41$, $p < .05$, males; $r = .46$, $p < .01$, females).

Mean level differences were found also when comparing the two social class groups. The mothers from the upper half of the SES distribution were rated significantly higher on variables dealing with interest in affectionate contact and social interaction with the infant, stimulation of the infant, and identification with the father. The women from the lower half of the SES distribution were rated higher on variables measuring depression during

pregnancy, apprehension over health and wellbeing of the infant, and degree of generalized apprehension. On a selected sub-set of home observation variables the upper SES subjects (females only) had significantly higher scores on variables assessing both Infant Smiles and Mother Smiles.

Minimal sex differences were observed for this sample. A higher rate of vocalizations among the males was one exception. Vocal behavior, in part, is considered to reflect arousal level and this finding was interpreted as being consistent with the results of other studies in which males exhibited higher arousal levels.

This is the final report of this project.

Publications:

Moss, H.A.: Infancy: Personality Development. In Wolman, B.B. (Ed.): International Encyclopedia of Neurology, Psychiatry, Psychoanalysis, and Psychology. New York, Van Nostrand Reinhold, 1977, Vol. 6, pp. 42-46.

Moss, H.A. and Jones, S.J.: Relations between maternal attitudes and maternal behavior as a function of social class. In Leiderman, P.H. and Tulkin, S.R. (Eds.): Cultural and Social Influences on Behavior in Infancy and Early Childhood. Stanford, Stanford University Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00230-11 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Intellective Processes in Young Children: Relationship to Cognitive Functioning in Infancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. J. Caron	Research Psychologist LDP NIMH
Other:	Rose F. Caron	Adj. Prof., Dept. of Psychol., Geo. Wash.Univ.

COOPERATING UNITS (if any)
George Washington University

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.10	.05	.05

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to examine the relationships between discriminative mnemonic processes in early infancy and intellective functioning in childhood. The cognitive performance of 4 to 7 year olds who represent behavior extremes on habituation procedures administered to these same samples at 3 to 5 months of age are currently being compared.

Project Description:

Although the measurement of mental abilities in infancy has undergone many changes and refinements and the available tests have some diagnostic value in the assessment of deficits, infant test scores have generally not been predictive of later intellectual functioning. General dissatisfaction with the absence of reliable age-to-age correlations of infant tests has led many to question their usefulness as a psychometric assessment device on the grounds that the abilities and skills which are tapped are largely sensory-motor and too remote from cognitive processes. Experimental investigators of infant learning and cognition, however, continue to be impressed with the range of individual differences in performance and with the possiblity that these early differences may prove developmentally stable and predictive of future accomplishment. For example, empirical observations suggest that response decrement to repeated stimulation (rate of habituation) in young infants may be related to performance on cognitive tasks such as concept formation and to conventional IQ measures in the preschool years. The purpose of the present project is to determine the extent of the relationship, if any, between infant discriminative performance in habituation and learning paradigms and measures of intellectual ability in the 4 to 7 year period.

Data have already been collected on the visual processing of some 500 4-month-old and 200 5-month-old infants, who are presently about 4 to 6 years of age. The task involved habituation of visual fixation to regular and distorted versions of schematic drawings of a human face. It has been argued that habituation rate may reflect the speed with which the infant processes incoming information. While this statement may be generally valid, it is unfortunately the case that rate of habituation is also affected by such extraneous factors as organismic state, competing external stimulation (e.g., attraction of the hands), type of target stimulus (e.g., simple or complex), general task involvement, etc. Our initial approach to the problem, therefore, has been to select subsamples of infants who exhibited deviant habituation rates, i.e., who either failed to habituate to the least complicated face stimuli or habituated rapidly to the most complex faces. "Complexity" was determined partially on an objective basis, i.e., by the number of elements contained in the figure (some faces were distorted by eliminating features such as eyes, mouth, or hair) and partially on an empirical basis (by rank ordering the stimuli according to mean speed of overall habituation). Of the 17 faces employed in the infant study, six were eventually selected as least complex and eight as most complex. Infants whose fixation decrement was in the lowest percentile for the easy faces and in the highest percentile for the complex faces constituted the initial subject pool. This pool was further reduced by eliminating any infant whose state appeared to have been questionable (i.e., fussy or tired upon entering the situation, needed a pacifier, had a bowel movement, etc.) and whose initial fixation levels had not met a predetermined criterion (suggesting that they may not have been interested in the task). The resulting sample included close to 300 children, of whom about 150 were still in the Washington, D.C. area.

Thus far, almost 100 infants ranging in age from 4:4 to 7:1 have been tested on the Stanford Binet Intelligence Scale. The IQ's of this essentially upper

middle-class suburban group range from 83 to 151 (Mean: 116.0). Forty-five of these infants (24 boys, 21 girls) were slow to habituate to relatively noncomplex stimuli and 51 (17 boys, 34 girls) were rapid habituators to complex stimuli. The difference in mean IQ between these two groups, while in the correct direction, is not significant (114.2 vs. 117.5, respectively) with males and females showing comparable differences. About 25 more infants remain to be tested. If the present trends continue, then we plan to do the following: First, we shall look at sub-items of the Binet scale, paying particular attention to tasks involving visual analysis (e.g., copying figures, maze tracing, etc.). Secondly, we shall recruit the children who were in our remaining infant subgroups (slow habituators to complex stimuli, fast habituators to noncomplex stimuli) to determine whether predictive validity might perhaps be better within one set of stimuli than the other. Finally, we shall inspect other aspects of our infant data (e.g., magnitude of recovery to novel stimuli) to see whether these relate more strongly to childhood intellect or to other areas of childhood cognition (e.g., school performance).

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00231-03 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Assessment of Early Intellective Capacities in Normal Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. J. Caron	Research Psychologist	LDP	NIMH
Other:	Rose F. Caron	Adj. Prof., Dept. of Psychol.	Geo.Wash.	Univ.
	V. R. Carlson	Research Psychologist	LP	NIMH
	S. L. Friedman	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)
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1.05	.50	.05

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad aim of this project is to chart the course of infant perceptual/cognitive development in the earliest months of life, with particular emphasis on four functions--discrimination, memory, association, and abstraction--as they unfold primarily in the visual modality. Presently we are interested in (1) the refinement of infant discrimination assessment methods, (2) depth perception in 2-month-olds, and (3) the infant's ability to use visual stimuli as predictive cues.

Project Description:

The overall purpose of this project was to assess intellective development in early infancy, with particular emphasis on the following visual functions: (1) visual capacity (discrimination), (2) visual recognition (memory), (3) visual signaling (association), and (4) visual selectivity (attention and abstraction).

Study A. The effectiveness of the familiarization-novelty method in the assessment of infant discrimination.

The current view of young infants as "competent" organisms derives largely from methodological advances which have enabled us to expose their genuine capacities. Since the validity of our estimates of competence can be no better than the sensitivity of our procedures, continuous evaluation of these procedures is a necessity. The purpose of the present study was to probe the effectiveness of the novelty-familiarization technique which has recently been adopted in lieu of the conventional habituation-dishabituation paradigm to assess visual discrimination and recognition memory in infants. Examination of those studies which have employed this new method suggests that it underestimates the ability of infants under 4 months to detect subtle stimulus differences. The familiarization-novelty procedure differs from the conventional habituation paradigm in two respects: (1) relatively brief familiarization intervals are used during which response decrement (habituation) is not required and (2) the familiari stimulus is tested in paired-comparison with a novel stimulus rather than successively or over sequential trials. Both operations may be disadvantageous to the younger infant who may need more time to process the familiarization stimulus (particularly in the case of difficult discrimination problems) and who is also notably less efficient in comparative scanning (because of position bias).

In order to determine which aspects of the familiarization procedure might underlie its shortcomings with younger infants, two experiments were conducted in which groups of pre- and post-4-month-old infants (Total N = 256) were exposed to unidimensional discrimination problems of varying difficulty under conditions of brief and prolonged familiarization and using both paired- and successive-comparison tests. For both age groups, magnitude of novelty preference was a positive function of length of familiarization, ease of problem, and degree of habituation during familiarization, suggesting that insufficient processing time can mask the infant's ability to discriminate stimulus change. The performance of younger infants was constrained by three additional factors: (1) they are more position-biased than older infants and thus lose the advantage of the paired-comparison test, (2) they are more subject to attention tropisms elicited by specific stimuli, and (3) they take much longer to process stimuli. These limitations apply only to unidimensional stimulus discrepancies since strong novelty effects were obtained for both age groups on equivalent problems involving multidimensional differences.

Generally the findings support the conclusion that the familiarization-novelty procedure may not be the method of choice for assessing the discriminative capacity of infants under 4 months of age. They also suggest that we may be better advised to use some variant of an "infant control" habituation technique, wherein each child is habituated to the same steep level of decrement and where, in the absence of fixed trial lengths, the younger infant's tendency toward obligatory looking is less likely to interfere with his true responsiveness to novelty. Finally, this research underscores the importance of distinguishing between competence and performance, and of being cautious about ascribing lack of proficiency to infants when our estimates may simply reflect insensitive methodology.

This completes the present study.

Publications:

"Constraints on the use of the familiarization-novelty method in the assessment of infant discrimination." Child Dev., 48: September 1977.

Study B. Depth perception in the young infant: a study of shape constancy.

Humans are primarily visual organisms and, given the central importance of human development of the visual system, the question of when and how the infant's perceptual world becomes like that of the adult remains of genuine scientific and pragmatic interest. The central paradox to be resolved is the fact that perception is so much richer than its apparent cause--the momentary retinal image. This paradox is most strikingly exemplified by the phenomenon of space perception, since we are able to see one more spatial dimension (depth) than is contained in the retinal stimulus. Moreover, we preserve the constant shape and size of an object regardless of changes in the retinal image which are produced by altering viewing distance or orientation in the third dimension. With increased experimental access to the infant's visual world, the long-standing controversy over whether man is genetically endowed with the capacity to see depth or whether it is acquired through experience (by correlation of visual cues with tactual-kinesthetic-proprioceptive feedback accompanying movement in space) can now be tested.

Initial experiments on depth perception and spatial constancies conducted by T.G.R. Bower indicated that infants as young as 2 months respond appropriately to object distance and thus preserve the constant shape and size of objects despite transformations in the third dimension. However, recent attempts to replicate Bower's depth perception studies have been unsuccessful and indicate that young infants may be responding to differential depth cues per se rather than real objects in space. The contradictory findings suggest that it might also be advisable to reexamine Bower's constancy research, particularly in view of its various internal inconsistencies.

The present study constitutes a further probe of the infant's ability to perceive constant shape (i.e., the ability to see invariant real shape despite rotation in the third dimension which alters retinal or projective shape). Using recovery from habituation to index discrimination, an initial experiment tested groups of 80-day-old infants (total N=120) in one of six treatment conditions. While the same figure served as the recovery stimulus in all conditions, the to-be-habituated stimulus differed between groups according to whether it deviated from the standard in terms of various combinations of objective shape, projective shape, and slant. The rank order of the groups on recovery was discrepant with Bower's results and implied that infants are indeed discriminating only the proximal cues specifying slant rather than real shape-at-a-slant. A second experiment involving 60-day-olds (N=20) and an additional 80-day-old group (N=18) indicated that the findings could not be attributed to sampling error, the age differential between Bower's subjects and our own, or tendencies to view the slanted figures projectively.

Overall, the results suggest that Bower is partially correct and partially incorrect in his claims for the three-dimensionality of early infant perception.

He is correct in his assertion that young infants do not see momentary retinal images, but he has probably overstated the case in his contention that they see space in a functionally significant way. From the present study and other recent research on the infant's ability to respond appropriately to depth, it appears that very young infants perceive the visual cues which specify depth (e.g., motion parallax and vinocular disparity cues) but have not yet become able to interpret this optical information in terms of the third dimension. As such, experience and/or maturation may still contribute to perceptual development (by coordinating space-related visual cues with those from other modalities), and while the young infant's visual world may not be a buzzing confusion, it has not yet become integrated into a stable, three-dimensional representation of reality.

The present study is now completed.

Publications:

"Do infants see objects or retinal images? Shape constancy revisited." Submitted for publication to Infant Behav. and Dev.

Study C. The infant's ability to extract information from the environment using visual stimuli as predictive cues.

Perhaps no aspect of early learning is more important than learning that some events regularly precede others and hence may be used to predict their occurrence. Although evidence of such predictive learning is commonly observed in the nursery (e.g., anticipatory sucking at the sight of the bottle) there has been little systematic study of this ability. The paucity of research on this question attests mainly to the complex methodological problems confronting the investigator who attempts to apply standard discrimination paradigms to a perceptually and motorically immature organism. In our laboratory we have developed a technology that has enabled us to condition infant behavior rapidly and to maintain it over multiple sessions. There is no question that 3 to 4-month-old infants can learn to modify their behavior as a function of programmed consequences, but we were unable to obtain solid evidence that they can learn to respond appropriately to differential cues. The conventional procedures presented undue difficulty and the results were seldom unequivocal. We were convinced that the standard experimental arrangements were somehow masking the infant's genuine ability to detect environmental regularities and that if we tailored our procedures more closely to the child's natural endowment, rapid and robust discriminative learning would follow. We attempted, therefore, to enhance the ecological relevance of the learning situation by (1) emphasizing correct choice rather than rate of response (shifting to a discrete-trial task), (2) making the response-reinforcer relationship less arbitrary (using a spatially differentiated response that was more integral to consumption) and (3) facilitating the infant's utilization of cues (localizing the discriminanda in space).

In brief, the infant, lying supine in a crib, was successively exposed to either of two audiovisual stimuli which were differentially positioned overhead. In the presence of S_1, which was located forward of his line of sight, he was required to make a large orientation to the right, whereupon he was rewarded with a kaleidoscopic display of colored lights and music on his right. In the presence of S_2, which was located to the rear of his visual field, he had to make a comparable orientation to the left in order to receive the same reward on the left. The stimuli, the response, and the reinforcement, thus were all topographically differentiated. Thirty-two (32) infants completed three or four discrete-trial discrimination learning sessions. Following brief training of left and right head turning behavior, the infants were given a minimum of 24 random test presentations of the discriminative stimuli. The results, in terms of speed and accuracy of learning, were impressive. Almost the entire sample (28 infants) exhibited significant levels of correct responding (first choices) on the random test trials, with half of the subjects scoring at least 80% on the second test day. The facility with which the infants acquired the discrimination under these conditions is consistent with animal research demonstrating the presence of constraints on the generality of learning. The results suggest that human infants, like other species, may have a natural predisposition to associate certain classes of stimuli with certain

responses, e.g., that topographical differences in stimuli, as opposed to qualitative differences, promote the learning of a spatial discrimination. Furthermore, under less artificial conditions, infants display behavior which has all the characteristics of problem solving. They not only employ response strategies but also appear to understand the predictive (signalling) function of the discriminative stimulus. This was evident in their careful inspection of the discriminanda before they behaved and in the smoothness with which they executed long runs of consecutively correct responses. Finally, in view of the remarkable performance of individual infants, it was impossible not to entertain the hypothesis that individual differences in this behavior might be predictive of future cognitive proficiency. A properly constructed discrimination learning task, thus, might provide a useful lens for examining the emergence of higher-order functions in early life and for assessing their developmental stability.

The present project has been completed.

Publications:

"Effects of ecologically relevant manipulations on infant discrimination learning." Submitted for publication to Infant Behav. and Dev.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00232-03 LDP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Antecedent Risk Factors and Infant Perception/Cognition: Effects of Obstetric Medication

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.L. Friedman	Psychologist	LDP NIMH
OTHER:	A.J. Caron	Research Psychologist	LDP NIMH
	Y. Brackbill	Prof., Dept. of Psychology	Univ. of Florida
	R.F. Caron	Adj. Prof., Dept of Psychology	Geo. Wash. Univ.

COOPERATING UNITS (if any)

8 hospitals in the Washington, D.C. Metropolitan Area

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.55	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There is evidence to suggest that medication administered to mothers during labor and delivery affects the newborn's ability to process environmental stimuli. The present study asks whether obstetric birth medication is a long-term risk factor for visual processing as it is for auditory processing. The study also asks whether various types of premedication and medication given during labor and delivery have different long-term effects on visual processing of infants. Four- and 5-month-old subjects who had previously participated in a study involving habituation of fixation to schematic faces, were grouped according to the medication their mother received: general, regional, or no anesthesia; analgesia; oxytocin. The groups ranged from full pre-medication plus general anesthesia to no premedication or delivery medication (natural childbirth). It was found that at 4 months of age analgesics and oxytocins are associated with slower processing. These results are less strong than those linking obstetrical medication to changes in auditory processing, thus raising the need for research exploring intersensory comparisons. The results also suggest it may be fruitful to examine inter-drug comparisons and age comparisons for the same modality.

Project Description:

Several studies have demonstrated both immediate and long-term delivery medication effects on infants' ability to process auditory stimuli. The present study asks whether obstetric birth medication is a long-term risk factor for visual processing as well. It also asks whether various types of premedication and medication given during labor and delivery have different long-term effects on visual processing of infants.

One hundred and ten infants were drawn from a larger sample of normal full-term 4- and 5-month-olds who had participated in a study by the Carons. There were fifty 4-month-olds and sixty 5-month-olds. These infants are a subsample from 400 infants on whom there is reliable data on visual habituation. The subsample represents extremes of the habituation continuum. All the subjects were normal, full-term children who had been alert, attentive and relaxed during the experimental session.

The subjects were classified into groups on the basis of the type of delivery medication (anesthesia) and premedication (analgesia, oxytocin) administered to their mothers during labor and delivery. Except for oxytocin, these medications have been previously implicated as depressants of newborn alertness. The effects of oxytocins on the psychological functioning of infants have not been studied before.

The major medication groups were: (a) General anesthesia; (b) Regional anesthesia; (c) No anesthesia. The major premedication groups were: (a) Analgesia; (b) No analgesia; (c) Oxytocin; (d) No oxytocin.

For the purpose of some analyses the above groups were divided into additional subgroups. These were: (a) General anesthesia with and without analgesia; (b) Regional anesthesia with and without analgesia; (c) No anesthesia with and without analgesia; (d) General anesthesia with and without oxytocin; (e) Regional anesthesia with and without oxytocin.

General anesthesia was given by inhalation using Nitrous Oxide, Ethylene, Methoxyflurane and Thiopental either alone or in combination. The regional anesthesia most commonly used (90% of cases) was lumbar epidural, either in a single dose or in a continuous manner.

The analgesia most commonly given (88% of the cases) was Meperidine. The mean dosage was 83 mg with a range of 50 mg - 200 mg. The oxytocin most commonly given (80% of the cases) was Pitocin.

The visual behaviors of the subjects were observed while they were seated in a dark viewing chamber looking at slides. The slides were rear projected on a 7 x 7 inch screen which was 13 inches away from the infant's eyes and in his (her) line of regard.

Each infant was exposed to two abstract art slides and to one schematic drawing of a female face. The first two slides served to familiarize the subject with the procedure and materials. The face slide was presented repeatedly for six 30-second trials.

Three measures of visual attention were taken during each of the face presentations. (a) First-Fixation---the length of the first look directed at the stimulus. (b) Total-Fixation---the total time of looking at each of the 30-second presentations. (c) Number-of-Fixations---the number of looks that the infant directed at the stimulus during each presentation. With repeated exposures of a stimulus, the magnitude of the first two measures are known to decrease while the magnitude of the last measure is known to increase. These changes in response magnitude over trials were taken to indicate attention decrement.

The data were analyzed to find out if obstetric medications affected (a) rate of attention decrement over repeated exposures of a stimulus, and, (b) amounts of looking over repeated exposures.

Infants in all the groups showed decrement in visual attention over trials. Analyses which held anesthesia constant show that analgesics and oxytocics are associated with less attention decrement than that evidenced in the absence of such drugs. The group comparisons did not show consistent differences in the amount of looking given by the various groups across trials. They did, however, raise the possibility that general anesthesia enhances irritable visual attention more than regional anesthesia does. The effects of obstetric medication on visual attention were found more often in the 4-month-olds' data.

These results are clearly less strong than are those linking obstetrical birth medication to changes in auditory processing. The interpretation of the data focusses on this inter-sensory comparison in conjunction with the differential rates pre- and postnatally at which visual and auditory systems mature both structurally and functionally. Across species research shows that the auditory modality is ontogenetically older than the visual modality. If at birth the more developed modalities lack in plasticity relative to the less developed modalities, then it is possible that the less developed modalities are better able to overcome the effects of obstetric medication or the effects of asphyxia enhanced by such medication. The results raise the need for research exploring the relative impairment caused by types of obstetric medications to different sensory systems as well as the persistence of such impairment.

This project has been completed.

Publication:

Friedman, S.L., Brackbill, Y., Caron, A.J., and Caron, R.F. Obstetric Medication and Visual Processing in 4- and 5-Month-Old Infants. Merrill-Palmer Quarterly, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00233-10 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Effects of Early Visual Experience on Perceptual/Cognitive Development in Infancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. J. Caron — Research Psychologist — LDP NIMH

R. F. Caron — Adj. Prof., Dept. of Psychol., Geo. Wash. University

COOPERATING UNITS (if any)
George Washington University

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION
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INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.25	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general purpose of this project is to examine the effects of specific conditions of early visual experience on subsequent mastery of particular discriminations. Specifically, we are interested in the relative influence of simple visual exposure to stimuli vs. "functional" exposure (stimuli correlated with differential consequences) on the young infant's later ability to differentiate these same stimuli. Also of interest is the contribution of active instrumental responding vs. "passive" responding in the functional setting.

PHS-6040
(Rev. 10-76)

Project Description:

Although research on perceptual-cognitive development in infancy continues to flourish, much more effort has been expended on the description of age-related capacities than on the determination of antecedent experiential factors which might influence perceptual change. The few attempts which have been made to manipulate the infant's early experience have been concerned primarily with the facilitative effects of broad "enrichment" operations on general aspects of sensory-motor responsiveness. As such, and because these studies have typically been conducted without recourse to stringent controls or to precise management of the infant's interaction with the supplemental environment, they have added little to our understanding of the mechanisms governing early cognitive development.

The major objective of this project is to examine the effects of very specific conditions of early visual experience on the infant's subsequent discriminative competence. In particular, we are interested in the relative influence of simple visual exposure to stimuli vs. "functional" exposure to them (i.e., where the stimuli serve as signals for the occurrence of important future consequences) on the infant's later ability to perceive differences between the stimuli. The fundamental theoretical issue here is whether incentives intrinsic to perceptual activity (e.g., the need to disambiguate the environment) are sufficient to guide discriminative development or whether perceptual learning is dependent on the total adaptive context in which behavior occurs.

The experimental strategy, as originally conceived, would have involved exposure of groups of 4-month-old infants to various discriminanda (shapes and patterns) previously demonstrated to be non-discriminable at this age, either in themselves or as the distinguishing features of more complex figures in which they were embedded. The conditions of exposure were to vary between groups in terms of (a) whether the stimuli had predictive validity or not (i.e., whether they signalled distinctive or nondistinctive forthcoming reinforcing events) and (b) whether the infant was active or passive in the situation (i.e., whether he must behave to produce the event or not). Upon terminations of training all groups were to be tested by means of familiarization novelty techniques to assess their ability to discriminate the previously exposed figures.

Progress on this project has been hampered by unforeseen methodological problems having to do, first, with the extraordinary difficulty encountered in teaching young infants to respond to visual stimuli as cues for differential behavior and, secondly, with the apparent insensitivity of certain familiarization-novelty techniques to the infant's genuine ability to discriminate subtle stimulus differences. Recent work addressed to these issues is decribed in Project Z01 MH 00231-03 LDP. While my collaborator, Dr. Rose Caron, will return to our original question in the light of these findings, my formal involvement in this study will be confined to integrating the information with Project Z01 MH 00234-01 LDP which is concerned with the development in normal and deviant infants.

This is the final report on this study.

Publications:

See Project Z01 MH 00231-03 LDP

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00234-03 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Infant Conditions the Mother: Directions of Influence in Mother-Infant Interaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Gewirtz	Research Psychologist	LDP, NIMH
OTHER:	E. F. Boyd	Assoc. Investigator	Univ. of Maryland Baltimore County
	G. Y. Browne	Assoc. Investigator	Virginia Div. of Youth Services
	C. L. Poulson	Assoc. Investigator	Princeton Child Development Institute

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.70	.80

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Several experiments were carried out on directions of influence in mother-infant interaction. Seemingly responsive but incidental behaviors attributed to each mother's own 3-month-old infant were found to condition maternal verbal phrases accompanied by smiles, smiles, nonsmile facial expressions, and the display of a particular toy. These demonstrations of the infant conditioning its mother are of one mechanism whereby the infant influences its mother in the early months of its life.

Project Description:

In recent years, there has been an increasing concern that observed relationships between parent and child behaviors are due not simply to the effect of the parent's behaviors on the child's behaviors, but reflect a two-way flow of influence between caregiver and child. This mutual-influence conception has provided the basis for these studies of sequential mother-infant interchanges. The instrumental-learning paradigm has been employed in an experimentally-contrived mother-infant interaction context. The questions asked are (a) whether a directed response (a vocalization or a headturn) that a mother believes her infant has emitted to her will influence (i.e., condition) an expressive or vocal response she has been emitting to it, possible without her even being aware of the process whereby her behavior is being influenced; and (b) whether a feature (the speed) of the headturn response of her infant that a mother is asked to condition will influence the response she selects to make to her infant.

In an interactive context, three experiments were carried out. In the first two studies, each of eitht mothers was asked to serve as "experimenter" in an investigation of whether infant "subjects" could learn that their behaviors can have social consequences. Each mother was asked to provide these consequences by saying a short phrase immediately following representations of one of her infant's behaviors. Mother and infant were on opposite sides of a one-way mirror, the mother facing the mirror side. All infant behaviors were simulated. A seemingly responsive but incidental behavior, attributed to the mother's own infant, was identified for her as merely occurring in the situation. An explicit attempt was made to give her no indication to attend to that incidental infant behavior, much less to foster it. That infant behavior was then presented contingent on one of the mother's verbal and/or expressive responses. The actual purpose of the experiments was to determine the reinforcing efficacy for everyday maternal social responses (verbalizations, facial expressions) of those seemingly responsive but incidental infant behaviors (vocalizations, headturns).

It was found that the simulated infant vocalizations (in Experiment 1) and headturns to mother (in Experiment 2) functioned as reinforcers to condition the mothers' verbal phrases and/or expressive responses. Hence these social responses of the mothers came readily under the control of what, in the interactive context, appeared to be the responsive vocal or headturn behaviors of their own infants. Systematic post-experimental interviews and unsolicited comments during the course of the studies suggested that this learning occurred without the mothers being aware either that (a) there were infant vocal or headturn behavior contingencies in effect for their verbal and/or expressive responses, (b) their responses had been conditioned, or (c) the behaviors of their infants had been simulated. These conclusions received support also from the fact that, in Experiment 2, expressive responses were conditioned even though mothers were not asked to make them.

Experiment 3 expands upon the first two designs by bringing the infant into the setting. The subjects were three mothers who had just conditioned their

infant's headturns by displaying toys contingent upon them. There was no indication to the mothers that the focus of experimental interest had switched from the infant's responses to the mother's. The mother sat behind a shaded window, which she raised to show her infant one of two toys when signalled that he had turned his head. The mother's presentation of one of the toys was followed by a signal that her infant had turned his head again within 4 seconds after the mother had lowered the shade. When she had presented the nontarget toy, the next headturn signal followed after 8 seconds. It was found that simulated infant headturns that more rapidly followed the mother's selective toy-display response functioned as reinforcers to condition that response.

In the child's formative years, the reciprocal-influence process of caregiver and infant upon each other's behavior promises to provide an important key for understanding the end products of adult-infant interaction and the adaptive learning of child and of caregiver. The principles identified in these experiments provide a basis for understanding of aberrations in learned outcomes, and hence can be of use in developing corrective changes for both parent and child components of their mutual interaction. Moreover, the fact that maternal expressive responses could be conditioned readily provides an important basis for understanding how such adult responses come under environmental control.

This project has been completed.

Publications:

Gewirtz, J. L., and Boyd, E. F.: Experiments on Mother-infant Interaction Underlying Mutual Attachment Acquisition: The Infant Conditions the Mother. In Alloway, T., Pliner, P. and Krames, L. (Eds.), Attachment Behavior. New York and London: Plenum Press, 1977. Pp. 109-143.

Gewirtz, J. L. and Boyd, E. F.: Mother-infant interaction and its study. In H. W. Reese (Editor), Advances in Child Development and Behavior. Vol. 11. New York and London: Academic Press, 1976. Pp. 141-163.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NO. (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00242-03 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (50 characters or less)
The Development of Sensitivity to the Feelings States of Others

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Zahn-Waxler	Research Psychologist	LDP, NIMH
	M. Radke-Yarrow	Chief, LDP, NIMH	LDP, NIMH
	R. A. King	Dir., Inpatient Service	Children's Hospital

COOPERATING UNITS (if any)

None

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.65	.50	1.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Very young children's comprehension of others' emotional states and their interactions with persons in distress were investigated. Twenty-four mother-child pairs were studied for a 9-month period. The children were 9, 15, or 21 months old at the beginning of the study. Trained mother observers recorded their children's responses to naturally occurring emotions and to simulated distresses. Between 1 and 2 years of age there were several significant developmental changes in children's reactions to witnessed distress in another person; with increasing age, children cried less, sympathized more, showed less amusement, more imitation, and slightly more aggression. A similar developmental progression appeared when the child was the cause of another's distress or injury. When children caused distress to someone, their reactions were sometimes intense and ambivalent and included self-punitive and reparative or remorseful responses as well. The findings begin to provide developmental norms for ho children are affected by emotions in their environments.

Project Description:

The moods and emotions of others are powerful sources of stimulation in the young child's environment. Others' expressions of pleasure, anger, fear, love, and sorrow, whether or not directed toward the child, provide contexts of early learning and experience. The focus of this research is on the development of very young children's responses to others' emotional experiences, principally in circumstances of distress. What is the young child's awareness and understanding of others' affects? What feeling do others' emotions arouse and how does the child interact with others in such affective encounters? In such engagements of child with others there are the raw materials for inquiry into the emergence of the child's empathic capabilities and compassionate behaviors.

Three age cohorts, of eight mother-infant pairs each, were selected at 9-10 months, 15-16 months, and 21-22 months of age. The subjects were observed over a 9 month period. The volunteer families were a middle to upper-middle class, suburban sample.

The mothers who had been given training in techniques of observation used a reporting guide for dictating accounts of naturally occurring events. These were events in which feelings or emotions were expressed by someone in the presence of the child. Events included emotions either caused or witnessed by the child. The mother also simulated feelings of fatigue, pain, affection, sadness, anger, coughing, enjoyment on a predetermined schedule. The circumstances of each affective event, the child's reactions to it, and responses made to the child were reported. At 3-week intervals an investigator visited the home, at which time s/he simulated affective incidents and observed the mother and child in interaction. Comparisons of mothers' and researchers' reports on children's reactions to simulated affect indicated generally high correspondence.

Children's reactions to others' emotions were categorized as : (1) no response, (2) attends, (3) cries or fusses, (4) imitates, (5) laughs or smiles, (6) seeks caregiver, (7) intervenes negatively by hitting or censuring, and (8) intervenes positively by physical contact, giving objects, expressing sympathy or comfort. With the exception of the first two categories, reactions to affect frequently occurred in combination, e.g., first imitating, then comforting an injured peer. There were no sex differences in any of these reactions to emotions. Even the youngest children in the sample were able to discriminate others' positive (e.g., laughter) and negative (e.g., crying, pain, discomfort) affect: When viewing someone in distress, children cried more, showed more sympathetic behavior, sought more contact with the caretaker, and laughed and smiled less than when the "other" expressed a positive emotion.

Multivariate analyses of variance indicated several significant changes in developmental progression in children's reactions to others' distress. Between the ages of 10 to 16 months, the predominant response form (other than simply attending to the event), was distress cries or general agitation

in the presence of another's emotion. Over the succeeding months children continued to show concern but became less overtly distressed by the other's distress; more interpersonal, sympathetic reactions directed toward the victim appeared and increased in frequency. The first forms of positive intervention appeared between 12 and 16 months. These tended to be physical contacts of patting or touching the victim with elements of both getting and giving comfort. By the end of the second year these contacts developed into more differentiated interventions (physical help, sharing, sympathizing, consoling). Several of the forms of positive intervention were initially learned through observations of others' interventions.

Other age changes in the second year indicated that children became progressively less amused by another's distress, were slightly more likely to imitate the other's emotion, and were somewhat more aggressive and negative toward the victim. The aggressive behaviors were considerably less frequent than the positive interventions.

Children not only had occasion to witness others' emotions of distress, they frequently caused injury (e.g., knocking over a peer, biting mother). Children's responses to caused events showed many similarities in developmental progression and in content to the child bystander events; for example, a decrease with age in distress cries and an increase with age in prosocial reactions (e.g., hugging their victims, expressing sorrow). There were new and exaggerated responses that appeared when the child caused distress; alternation of aggression and compassion, self-punitive behaviors, and, later, expressions of reparation and remorse. These responses suggest that children at this early age are beginning to distinguish when they are or are not responsible for another's distress. In their acts of reparation and comfort there may be the precursors of early conscience development.

This study is intended to begin to establish developmental norms (eventually for older children as well) for how children are affected specifically and directly by emotions of others. With such a normative base it should be possible better to assess deviations in emotional reactions of children and to assess the effects on children of various kinds of emotional experiences. In addition, the empirical data from this research contribute to theories of empathy, altruism, guilt and conscience development.

A manuscript is being prepared for publication.

Publications:

Yarrow, M. R. and Waxler, C. Z.: The Emergence and Functions of Prosocial Behaviors in Young Children. In Parke, R.D. and Hetherington, E.M. (Eds.): Child Psychology: Contemporary Readings. New York, McGraw-Hill Book Company, 1976, in press. Also in Smart, R.C. and Smart, M. (Eds.): Readings in Child Development and Relationships (1977) and Infants: Development and Relationships. (1978) New York, Macmillan Publishing Company, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00243-03 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Inferential Capabilities and Prosocial Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Zahn-Waxler	Research Psychologist	LDP NIMH
	M. Radke-Yarrow	Chief, LDP, NIMH	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	10	.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Relations between prosocial behaviors and children's abilities to take another person's visual and social-emotional perspectives were examined. Perspective-taking tests were given to 115 children between the ages of 3 and 7 years. Prosocial behavior was assessed in eleven experimental situations. Ability to take another's perspective increased significantly with age. When sex differences were present, girls were superior at perspective-taking. There was a relation between physical and social perspective-taking only for children 4½ years and older. In general, the ability to make inferences about another's experience was not associated with the inclination to act sympathetically on another's behalf.

Project Description:

The relation between positive social behavior (helping, sharing, comforting) and children's cognitive comprehension of the perspectives and experiences of others was investigated. Two associated questions were also examined: namely, when do children understand that the social and emotional perspectives of others may differ from their own, and what is the relation between understanding others' physical perspectives and understanding others' social and affective perspectives?

The subjects were 115 white, middle class children, ages 3 to 7 years, currently or previously in attendance at a suburban nursery school.

Eleven tasks designed to measure the child's ability to understand the points of view of another person were individually administered. The tasks included visual perspective-taking and social-emotional perspective-taking. The child's helping, sharing, and comforting behaviors were assessed in experimental situations and also observed in the natural setting.

The ability to comprehend another's roles and psychological states increased with age on each of the eleven tasks. When sex differences were present in development, the performance of girls was superior to that of boys. Physical and social perspective-taking were related only for the older children studied (the 4½- to 7-year-olds). The child who failed a perspective test often succeeded on a second trial. Thus, stable assessments of an inability to take the other person's perspective may require more than a single trial on experimental tasks. Except for the youngest children, there was no correspondence between cognitive perspective-taking and prosocial behavior. If one is to understand the origins and functions of altruistic behavior in young children, factors other than perspective-taking cognitive skill will need to be taken into account. For young children, comprehension of another person's emotional state is a poor predictor of the kinds of responses they will manifest in the presence of others' emotional needs.

This project has been completed.

Publications:

Zahn-Waxler, C., Radke-Yarrow, M., and Smith, J. P.: Perspective-taking and prosocial behavior. Dev. Psychol., 13: 87-88, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00244-02 LDP

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Behavior Profile for Child Psychopathology and Social Competence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Achenbach	Research Psychologist	LDP	NIMH
Other:	C. Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)
25 child guidance clinics and other child mental health agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	.35	.80

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the research is to develop a standardized behavioral profile for child psychopathology and social competence to be used for research on etiology, treatment effects, epidemiology, and prognosis, as well as for clinical purposes. Parents of disturbed and normal children fill out a behavior checklist describing their child's behavior problems and strengths. Data from the checklists are factor analyzed to identify items that cluster together within various age and sex groups. The clusters are employed as dimensions of the behavior profile. Empirically obtained distributions of scores on each cluster provide norms with which the scores of individual children can be compared, and behavior profiles obtained on the same child at different times can be used to assess changes in behavior.

PHS-6040
(Rev. 10-76)

Project Description:

An acute problem in research on the etiology, epidemiology, treatment, and prognosis of child psychopathology is the lack of a standardized and objective system for diagnosing and classifying childhood behavioral disorders. The purpose of the present research is to develop the Child Behavior Profile, an instrument that employs readily obtainable data to portray children's behavioral problems and social competencies in a standardized and objective format.

The data are obtained with the Child Behavior Checklist, which is filled out by parents of children aged 4 to 16 years. Behavior problems (e.g., hyperactivity, disobedience, fears, bedwetting) are scored on a three-point scale according to intensity and frequency. Social competence items include reports of participation in sports, hobbies, and organizations, special skills, number of friends and amount of interaction with them, and school performance. The Behavior Checklist is the product of several pilot editions that have been tried out in child guidance clinics and have been progressively revised on the basis of feedback from clinicians, parents, and paraprofessionals.

The data for the Child Behavior Profile have been obtained from Behavior Checklists filled out by parents of children seen in some 25 mental health settings, plus randomly selected parents of normal children. Factor analysis is employed to identify clusters of items that tend to occur together in children grouped by age and sex. Several hundred subjects are included in each sample (e.g., boys aged 6 to 11, girls aged 6 to 11, boys aged 12 to 16, etc.)

The clusters of items obtained through factor analysis are used to define the dimensions of the standardized profile for each age and sex group, and the empirically obtained distributions of scores on each cluster provide norms with which the scores of individual children can be compared.

The Profile can be used to classify children for purposes of research on etiology, treatment effects, epidemiology, and prognosis, as well as providing clinicians with a standardized tool for describing and communicating about psychopathology. Profiles have been completed for boys aged 6 to 11 and 12 to 16, with Profiles for girls aged 6-11, and 12-16, for boys aged 4-5, and for girls aged 4-5 expected to be completed in that order. Average one-week test-retest reliability coefficients have been .89 for 6- to 11-year-old boys and .82 for 12- to 16-year-old boys. Average interparent agreement coefficients have been .74 for 6- to 11-year-olds and .79 for 12- to 16-year-olds. Significant stability has also been found in Profiles scored before and after outpatient psychiatric treatment, with stability coefficients averaging .63 for 6- to 11-year-olds assessed at a 15-month interval and .54 for 12- to 16-year-olds scored at an 18-month interval. Q correlations which reflect the stability in overall Profile shape, averaged .64 and .68 respectively, in these two groups over the same periods. Thus, despite decreases in the number of behavior problems reported after treatment, the patterning of behavior remained relatively stable.

Publications:

Achenbach, T. M. The Child Behavior Profile: I. Boys aged 6 through 11. J. consult. clin. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00245-02 LDP

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)
Objective identification of the hyperkinetic syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T. M. Achenbach Research Psychologist LDP NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.05	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study is to identify objectively the behavioral patterns associated with organic characteristics found through intensive medical work-ups of children referred for hyperactivity. Each parent of a referred child independently completes a behavior checklist from which a standardized behavior profile is derived. Degree of inter-parent agreement is computed and the profiles are analyzed in order to classify children according to types of behavior patterns. Classification of children in this way is then compared with ratings of ward behavior and with the outcomes of the medical work-up to determine whether the behavioral typology corresponds to characteristics of the children identified clinically.

PHS-6040

Project Description:

The "hyperkinetic syndrome" is today probably the most widely publicized of childhood disorders. There has been a tremendous increase in the number of children labelled as hyperkinetic and a great deal of controversy over such diverse approaches to treatment as chemotherapy, dietary manipulations, behavior therapy, and laissez faire permissiveness. Etiological hypotheses show a parallel diversity, with minimal cerebral dysfunction, allergies, anxiety, inappropriate reinforcers, constitutional characteristics of temperament, and creative nonconformity all being implicated by one authority or another.

One reason for the diversity of treatments and etiological hypotheses is that there is no objective way to define the behavioral phenotype of hyperkinesis. Since most children are from time to time too active for the tastes of their parents and teachers, and since the term "hyperactivity" and even "hyperkinesis", has gained such wide currency, reports of high activity level alone cannot be regarded as pathognomic. Identifying children who share some common etiological factor with respect to activity level or who would benefit from a particular treatment has thus been made difficult by the excessively broad application of the concept of the hyperkinetic syndrome.

In order to determine whether there is an identifiable behavioral phenotype corresponding to a clinical syndrome which may include organic anomalies and which may benefit from chemotherapy, collaborative research is being carried out with Dr. G. L. Brown of the NIMH Biological Psychiatry Branch in connection with a study of children referred for hyperactivity (cf. Biological Psychiatry Branch Project Number Z01 MH 00100-03 BP). Both parents of each referred child independently complete the Child Behavior Checklist (described in report on Project Number Z01 MH 00244-02 LDP). An extensive life history is also obtained and the children are admitted to the NIH Clinical Center for several weeks of Medical and behavioral work-up.

Without knowledge of the results of the work-ups at the Clinical Center, we are scoring the behavior profile patterns from the checklist completed by each parent. Interparent reliability is assessed and the separate profiles as well as a composite based on the checklists completed by each pair of parents, are analyzed to identify particular types of profile patterns. These are being compared with the results of the clinical work-ups to determine whether the types of profile patterns correlate with differential clinical findings and with ratings of ward behavior. Preliminary findings indicate a clearcut behavioral phenotype of hyperactivity and high correlations between parent ratings on our Checklist and one developed by C. Keith Conners for use in evaluating the effectiveness of drug treatments with children.

If further relationships between the behavior profile patterns and clinical findings are confirmed, the profile patterns can be employed to form homogeneous groups for research purposes. As specific etiologies and/or treatments are discovered for these groups, the profile patterns can be employed by

clinicians to guide diagnosis and the choice of treatment.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00246-02 LDP

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Follow-up Study of Children Differing in Behavior Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Achenbach	Research Psychologist	LDP NIMH
Other:	C. Edelbrock	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)
3 Child Guidance Clinics in New Haven and Waterbury, Connecticut

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.20	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the research are (1) to determine the stability of behavior profiles based on behavior checklists describing disturbed children, and (2) to determine the prognosis for children differing in initial profile patterns. Parents of children for whom Child Behavior Checklists were filled out at intake filled out Checklists describing their children's behavior problems and social competencies following treatment. Behavior profiles based on the follow-up Checklists are being compared with those based on the initial Checklists to determine the stability and differential prognosis for different types of profiles in various age, sex, and socioeconomic groups.

PHS-6040
(Rev. 10-76)

Project Description:

Effective utilization of mental health services to children is greatly handicapped by the current lack of knowledge about prognosis for various behavior problems. Although the demand for services to children far exceeds the supply, there is no objective basis for selectively assigning treatment to those who will benefit most, because it is not known which children will actually be helped by treatment, which ones will improve without treatment, and which ones will fail to be helped by current treatment approaches.

A primary obstacle to research on prognosis has been the lack of a standardized and reliable diagnostic-classification system for childhood behavior disorders. As described in the report on Project Number Z01 MH 00244-02 LDP the investigators have developed a standardized behavior profile for child psychopathology and social competence. It is expected that children having different profile patterns will differ in their prognosis under various conditions of treatment and nontreatment.

To test both the stability and the predictive power of the profile patterns, a follow-up was conducted of 200 children for whom Child Behavior Checklists were filled out in three clinics at intake. Parents of these children were asked to fill out Checklists describing their children's behavior problems and social competencies following termination with the clinics. Statistical analyses are now being conducted to determine whether children who differed in their initial profiles subsequently differ with respect both to their patterns of problems and competencies and to their general adjustment. Preliminary findings indicate statistically significant stability in the patterns of reported problems despite significant declines in the number of problems reported.

Further analyses are expected to show whether initial profile patterns are differentially related to treatment outcome and whether the patterns are useful for determining prognosis under typical clinic procedures. The findings will also provide the basis for future controlled studies of the effectiveness of various treatments with children having different profile patterns.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00247-02 LDP

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Relations between Cognitive and Behavioral Patterns in Children with Endocrine Abnormalities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Achenbach	Research Psychologist	LDP	NIMH
Other:	C. Edelbrock	Staff Fellow	LDP	NIMH
	R. Johnsonbaugh	Pediatric Endocrinologist	Nat. Naval Medical Center	

COOPERATING UNITS (if any)

National Naval Medical Center

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.10	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Behavior checklists and measures of cognitive functioning are being obtained on normal controls and children who have thyroid and sex hormone abnormalities in order to determine whether hormone levels affect patterns of cognitive and behavioral adaptation and the relationships between them. The study is designed to test the hypothesis that increases in sex hormone level affect patterns of cognitive functioning by improving automatization abilities at the expense of ability to engage in complex perceptual restructuring. It is also designed to determine whether premature craniosynostonis noted in hyperthyroid children interferes with cognitive functioning.

PHS-6040
(Rev. 10-76)

Project Description:

One of the most neglected aspects of research on childhood behavior disorders is the interface between cognitive functioning and maladaptive behavior. Research and theory tend to focus on cognitive disabilities as one substantive area and "emotional" problems as a separate substantive area. However, from a developmental perspective, cognitive functioning is intimately interwoven with the social, biological, and emotional aspects of adaptive processes. On the one hand, a child's level of cognitive development is likely to set limits on the types of adaptive strategies available to him. On the other hand, social, biological, emotional, and motivational factors are likely to influence the degree to which a child makes use of his cognitive capacity.

Work by other investigators has suggested that sex hormone levels affect the action of neurotransmitters in the brain, and that these, in turn, affect the patterning of cognitive abilities. In particular, it has been found that high levels of sex hormones, especially estrogens, are positively correlated with performance on automatization tasks requiring quick, accurate responses, but are negatively correlated with performance on tasks that require complex perceptual restructuring. Our study is designed to test this hypothesis by comparing cognitive functioning and behavioral patterning before and after treatment in children who receive sex hormone supplementation for various endocrine abnormalities. Children with thyroid abnormalities are also being studied because clinical observations have revealed premature craniosynostonis in these children,and it is important to determine whether cognitive and behavioral patterns are being affected by this condition. The children and their sibling controls are recruited by Dr. Roger Johnsonbaugh through his pediatric endocrinology service at the Naval Medical Center.

Our measures of cognitive functioning include the Wechsler Intelligence Scale for Children (WISC), the Embedded Figures Test, a measure of speed of color naming, and a measure of speed of object naming. Differences between perceptual restructuring measures (WISC, Block Design, Embedded Figures Test) and automatization measures (color naming, object naming) will be compared at two testings to determine whether changes in hormone levels after medication cause changes in cognitive patterning that do not occur in normal, unmedicated control subjects.

Preliminary findings indicate some differences in cognitive patterning between children with thyroid abnormalities and their normal controls, but not enough subjects have yet been tested to determine the generality of these differences or to draw conclusions about the effects of sex hormones.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00248-01 LDP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Sources of Variability in Children's Sensitivity to Others

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Barrett	Staff Fellow	LDP NIMH
	Marian R. Yarrow	Chief, LDP NIMH	LDP NIMH

COOPERATING UNITS (if any)

Green Acres Summer Day Camp

LABORATORY:

Laboratory of Developmental Psychology

SECTION:

INSTITUTE AND LOCATION:

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.85	.50	4.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to investigate individual differences in children's sensitivity to others, and to examine sources of this variability. The study involves the naturalistic observation of children at a summer day camp, as well as interviews with each child to study his/her social inferential abilities. Specific issues investigated include (1) relations between aggressive and assertive behaviors and prosocial behaviors, (2) the dimensionability of prosocial behavior, specifically relations among different types of prosocial acts, (3) the possible mediating effects of role-taking and inferential abilities with regard to the relations between children's past interpersonal experiences and their later social behaviors, (4) sequential dependencies involving aggressive and prosocial behaviors, i.e., the facilitative or inhibitory (accelerating or decelerating) effects of expressed aggression with regard to later prosocial behavior. In addition, sex differences in aggression and reaction to aggression as a function of mode of aggression, sex of target, and sex of initiator are investigated.

Project Description:

The present study examines sources of variability in prosocial behavior in young children. Proposed and approved as a continuation of an earlier study on the development of prosocial behavior (Project #73-M-02, Developmental Studies of Altruism), the current study focuses on research issues raised by results of the earlier study; specifically, questions concerning the dimensionality of prosocial behavior, behavioral correlates of prosocial behavior, and the role of social inferential ability in the development of prosocial behavior.

Subjects were 79 children ages five to eight, attending a summer day camp. Each subject was observed eight times over the course of the six week period, in both free play and camp activity. During each of the 15 minute observation sessions, observers recorded the children's social behaviors on a precoded form. Inferential ability was assessed in an individual interview, in which the child interpreted a series of videotaped social interaction.

In the last annual report, findings concerning correlations between prosocial behavior and aggression were reported. In addition, we summarized results of an analysis concerning the mediating role of social inferential ability in the prediction of prosocial behavior.

This year data analyses have focused on three issues: a further examination of the role of inferential ability in the development of prosocial behavioral competencies; sequential constraints in children's social interactions, particularly with respect to prosocial and aggressive behaviors; and sex differences in aggression and reactions to aggression. Results relating to the first two issues are available and are summarized below.

The results of our study have not shown a direct link between the ability of children to make inferences about others' feelings and behaviors and their capacities for prosocial intervention. However, the data indicate that this dimension of cognitive functioning may nonetheless play an influential role with respect to the children's prosocial activity. Analyses have indicated a significant interaction between social inferential ability and interpersonal assertiveness in the prediction of children's helping, comforting and sharing. Specifically, it appears that for children who are highly assertive, increased sensitivity to others' behaviors and emotions is positively related to frequency of prosocial behaviors. On the other hand, for children who are more timid and unassertive, relations between social inferential ability and prosocial are negative. The latter relationship suggests that increased awareness of the other's situation may result in greater emotional arousal, which may serve to inhibit rather than facilitate prosocial intervention in the uncertain child.

Sequential analyses have indicated significant regularity in young children's behavior episode patterns. For example, following the expression of aggression or the receiving of aggression, the child is relatively unlikely to initiate prosocial activity in immediately subsequent

social encounters. Conversely, the likelihood of prosocial activity is significantly increased by the expression of prosocial behavior in the antecedent episode. In addition, and consistent with previous research, our findings indicate that aggressive episodes are more likely following other aggressive episodes than would be predicted on the basis of unconditional probabilities. Similar patterns of behavior sequences have been identified for both boys and girls in the sample. Results are currently being evaluated in conjunction with our correlational findings concerning frequencies of prosocial and aggressive activity.

Analyses relating to sex differences in aggression are just beginning. It should be noted that the analyses focus on issues that have not been given much attention in the previous literature on sex differences in aggression, specifically: sex differences in aggression with respect to both modality of aggression and target of aggression, sex differences in frequency of aggression relative to the totality of the child's social interaction, and differences in reactions to aggression as a function of sex of target and sex of initiator. These analyses will help to elucidate previously reported findings relating to sex differences in aggression, and will further our understanding of the socialization of aggression and prosocial activity in young children.

Publications:

Barrett, D. E. & Radke-Yarrow, M. Prosocial behavior, social inferential ability and assertiveness in children. Child Dev. 48; 475-481, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00249-02 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

EEG Visual Evoked Potentials (VEP) Components Within and Across Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. L. Friedman	Psychologist	LDP NIMH
OTHER:	A. B. Barnet	Assoc. Prof. Dept. of Neurology & Child Health & Development	Geo. Wash. Univ. School of Med. & Health Sciences
	I. P. Weiss	Asst. Prof. Lecturer, Dept. of Physiol. & Child Health & Development	Geo. Wash. Univ. School of Med. & Health Sciences
	E. S. Ohlrich	Medical Student	Univ. of Wisconsin Madison, Wisconsin

COOPERATING UNITS (if any)
George Washington University School of Medicine and Health Sciences

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.25	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project describes maturational changes in the complexity and latency of EEG visual evoked potentials recorded from 16 normal children who were tested repeatedly over a period of three years. Between two and fourteen components were identified in the different VEPs. The number of the components increased and their latency decreased with age. The change in the latency of the components was non-linear, most of it occurring in the first three months of life. While short-latency components (appearing in the first 100 msec. after stimulation) remained relatively constant across age, long latency components progressively approached latency values generally reported for adult VEPs. Individual differences were found in the complexity of the VEP configuration, in the components which were present in a child's VEPs,and in the direction and degree of age related latency changes. No effects due to sleep state, sex and race were found.

PHS-6040
(Rev. 10-76)

Project Description:

This project describes maturational changes in the complexity and latency of EEG visual evoked potentials recorded from 16 normal children who were tested repeatedly over a period of 3 years. The children were tested between 7 and 17 times at approximately bi-weekly intervals until six months, at three month intervals from six to eighteen months, and then at six-month intervals.

The stimulus set consisted of 100 intense white light flashes presented at interstimulus intervals of 2.5 seconds. The EEG derived from an electrode at O_z referred to joined mastoids was averaged over the stimulus set to yield each VEP. A one second post stimulus analysis time was used.

The minimum number of VEP components identified was 2 and the maximum 14. For each child, VEP components were identified with considerable confidence across age, and components of the same polarity and of similar latency range appeared in the data of the different children.

The number of components in each individual's VEPs increased with age. The VEPs recorded between birth and seven months of age were significantly less complex than those recorded at later ages. In general, component latency decreased with age. The change, however, was not linear: Most of the change occurred in the first three months. Components in the first 100 msec. showed relatively less age related latency change than later components.

Individual children varied in the mean number of components which could be identified in their VEPs. Likewise, children with the same mean number of components did not necessarily have VEPs with identical components. Children also varied in the degree to which their VEP components changed with age, and there were individual differences in the direction of the latency changes with age. Occasionally, instead of the typical decrease in latency with age, there was an increase for one or more components in a given child.

Most studies of VEP changes during infancy have reported quantitative information about one or two VEP components. The longitudinal research design and the sampling of 1000 msec. after stimulation which were used in the present study permitted the elucidation of many VEP components. While these components differed in reliability and stability over time, they appeared to be useful for characterizing the VEP. Age related decreases in latency and an increase in the complexity of VEPs have been reported previously, but our finding concerning the differential course of age related change in early and late components has not been previously described.

Neurophysiological research has suggested that short latency VEP components are the product of brain activity in the specific sensory cortex which is relatively mature at birth while longer latency components represent activity in later to develop brain areas and in areas which develop increasing connections during maturation. Our findings may reflect the differential development of specific sensory and perceptual function in the first three years of life.

This is a final report. A manuscript is being submitted for publication.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT USE THIS SPACE)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00250-02 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Information Processing in Premature and Full-Term Infants at the Perinatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.L. Friedman	Psychologist	LDP NIMH
OTHER:	B.S. Jacobs	Psychologist	LDP NIMH
	M.W. Werthmann	Chairman, Dept. of Perinatal and Neonatal Pediatrics	Washington Hospital Center Washington, D. C.

COOPERATING UNITS (if any)

Washington Hospital Center, Washington, D. C.

LAB/BRANCH
Laboratory of Developmental Psychology
SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.20	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study precursors of cortical function and temperament are compared in infants varying in gestational and medical status at birth. The infants to be studied will include healthy as well as previously sick prematures at their expected date of delivery, healthy full-term neonates and postmature neonates. Cortical function will be evaluated by measures of habituation, dishabituation and transfer of learning in response to tactile, auditory and visual repeated stimulation. Temperament will be assessed by measures of states of consciousness, the range and quality of activity while the babies are free of apparent discomfort and by the persistence of spontaneous and elicited crying in the face of experimentally designed attempts to quiet the infants. The results are expected to identify factors which place children at greater than average risk for later intellective and behavioral dysfunction. The results will also provide information about the importance of maturation and medical integrity for the function of different sensory modalities and the organization of behavior.

Project Description:

Most pregnancies last between 38 and 42 weeks. Some, however, terminate much earlier or later. Medical technology has made it possible for infants born as early as at 28 weeks or as late as at 50 weeks of gestation to survive. This is certainly an impressive medical accomplishment; yet, the psychological outcomes of too short or too long periods of gestation are not fully understood. It is not obvious that the central nervous system of a premature organism is ready to benefit from extrauterine stimulation. Nor is it obvious that a postmature organism has not suffered because of its not being exposed to an extrauterine environment at a possibly critical period for some aspects of development. Birth not at term is often but not always associated with medical complications. This raises the question about the role of illness in early development. It is possible that a newborn who spends much energy in a struggle for biological survival has very little energy left for psychological development.

Evidence from late infancy and childhood indicates that children who were not born at term are at a greater than average risk for intellective and behavioral deficits. In this study we explore some precursors of intellective and behavioral deficits in early infancy -- in the days after birth or at the expected date of birth (in the case of prematures). Knowledge of specific weaknesses and strengths at this early stage should make it possible rationally to plan early intervention programs to stimulate those aspects of development which show deficits due to birth not at term.

The psychological functions to be studied are of two classes: information processing as an index of the degree of cortical maturity and integrity and temperament. Information processing will be assessed in the tactile, auditory and visual modalities. In the examination for tactile information processing, the infant's cheek will be stroked repeatedly by one of two non-noxious stimuli (a nylon filament and a small brush) for 2-second periods, with a random interstimulus interval of 15 to 25 seconds. When the baby has habituated to a predetermined criterion, the other stimulus will be applied until the baby habituates to it. The data will provide measures of habituation, response to novelty (dishabituation) and a comparison of the ease of habituation on the second occasion relative to the first (transfer of learning). In the auditory information processing examination the infant will be presented with one of two tones (500 cps; 1500 cps at 80 dB). The procedure and measures will be equivalent to those described for the tactile modality. In the visual information processing examination each of the two stimuli (revolving, lighted, differently colored plexiglass boxes) will be presented for 30-second periods starting with the infant's first look at the stimulus. The inter-stimulus interval will be 10 seconds. The procedure and measures will be similar to those employed in the tactile and auditory procedures.

Temperament will be assessed by the range and quality of the babies' behaviors when they are free of discomfort as well as by the resistance of their

spontaneous and elicited crying to comforting. The temperament assessment will be conducted when the baby is satiated and quiet. During 18 minutes the baby will be observed for periods of 5 seconds to be followed by 10-second periods in which the experimenter will note her observations. The observations will include the infant's state of consciousness, his/her body, limb, and facial movements as well as looking activity and vocalizations. Should the baby spontaneously cry, an experimenter will intervene in a progressively soothing manner in an attempt to comfort the baby. The intervention and the infant's responses will be coded. The temperament assessment will terminate with the coding of intervention following elicited crying. Crying will be elicited by flipping a finger against the infant's sole.

The procedures, stimuli, and equipment for the study have been tested. Data collection is now underway.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00251-01 LDP

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Contingent Maternal Responding on Infant Crying

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jacob L. Gewirtz Research Psychologist, Laboratory of Developmental Psychology, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.20	.20	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Will contingent maternal responding to nondistress infant crying lead to a subsequent decrease in that crying (as the 1972 Bell & Ainsworth conclusion has led many to expect) or will it lead to a crying-rate increase (as instrumental-learning theory would expect)? Subjects were 10- to 12-week-old normal infants. Infant lay on floor, alone in room with door ajar. The cry response (fuss or cry) unit was defined as a shrill or whiney vocalization, 5 sec. long, accompanied by a facial grimace. Under noncontingent responding, mother entered the lab routinely to visit infant, provided it was not crying. Under contingent responding, mother's visits were contingent on infant's 5-sec. cries. Number of maternal 10-sec. visits (i.e., stimulus density) was comparable under both treatments. Mother acted naturally, but did not pick up infant. The Contingent mean cries-per-minute(2.0) was found greater at $p < .01$ than the Noncontingent mean (0.1). Therefore, maternal responding contingent on infant cries increased the cry rate as expected under instrumental-learning theory. This result has implications for caregiver management of infant crying.

Project Description:

The purpose of the research is to study that portion of infant crying that is communicative and instrumental and not elicited by physical distress; in particular, to determine some features of a mother's responding to her infant's nondistress crying that foster or maintain that crying and those that minimize or preclude that crying. A second objective of the investigation is to use the findings to make recommendations to caregivers about constructive procedures for minimizing or precluding infant crying in the first year of life.

In early life, physical distress (e.g., hunger cold, pain) underlies the crying termed "unconditioned," "expressive," or "respondent." On that basis alone, infant crying has a unique potential for controlling caregiver behavior. However, there is no apparent unlearned basis for the infant crying termed "operant," "instrumental," or "communicative," which constitutes perhaps the most important feature of the communication of infants with caregivers and thus provides a key to understanding parent/caregiver--infant interaction and the earliest ways the infant learns to control the environment. Infant crying has also provided the most frequent basis for indicators of an infant's attachment to mother/caregiver. An understanding of how crying patterns are acquired offers a unique basis for understanding how attachments evolve in the child. At the same time, there has been recent controversy about how caregivers would respond to infant crying to alleviate bodily distress while not fostering crying and "spoiling" the child (Bell & Ainsworth, 1972; Gewirtz & Boyd, in press). Hence, it is important to understand the environmental bases of infant crying in early life, and knowing how to respond to such crying represents a management problem of great practical concern. This project was directed to determining whether and how operant/communicative infant crying/fussing could be controlled by maternal responding to it. Specifically, will contingent maternal responding to nondistress crying lead to a subsequent decrease in that crying (as the 1972 Bell & Ainsworth conclusion has led many to expect) or will it lead to a crying-rate increase (as instrumental-learning theory would predict)?

Mothers were asked to bring their infants to the laboratory daily at a time judged best for the infant. To preclude physical distress-induced crying, infants were studied only when they had been recently fed and diapered, had slept routinely, and did not appear tired or ill. The criterion cry response (fuss or cry) unit was defined as a shrill or whiney vocalization, 5 seconds long, accompanied by a facial grimace. Observer agreement on cries was high. Daily sessions were 16 minutes long, but were terminated prematurely when an infant cried/fussed without pause for 1 minute, or fell asleep.

With its mother not in view outside the experimental room, each infant was positioned on the floor, alone in the room, facing a wall, in which half way up there was a one-way mirror, and at the top of which there was a mobile hanging from the ceiling. Under noncontingent maternal responding, a mother routinely entered the lab room to visit her infant at one-minute intervals, provided the infant was not crying. Under contingent maternal responding,

each mother's visit began immediately after her infant had cried for 5 seconds. The standard maternal response was a 10-second visit to the infant. Each mother was asked to act naturally, by touching, nuzzling, patting, squeezing, kissing, and/or talking to her infant, but not picking up the infant. She was signalled to enter the room, and to leave it after 8 seconds of a visit had elapsed. Upon departing, she activated the overhead mobile gently. She routinely checked her infant's diaper and, if diapering was indicated, did so while the session-duration time was stopped. The number of maternal visits was comparable under both noncontingent and contingent treatments. Hence the outcome would not be explainable as due to the differential elicitation of crying.

Mothers and their 10- to 12-week-old (at the start of the study) normal, full-term, infants served as subjects. Seven subjects received first the noncontingent and second the contingent treatment; and 3 subjects received the treatments in reverse order. When the rate of crying had stabilized under the first treatment, the infant was shifted to the second. Thus, each infant served as his/her own control in a repeated-measures design. Excluded from the sample were infants exhibiting very-high, very-low, and very-variable crying rates during the first treatment condition. On this basis, it was necessary to discard two infants for every infant qualifying.

The dependent measure was the cry rate/minute per daily session (or treatment within session, when a treatment shift occurred during a session). The final session of the first treatment condition occurred when the cry rate/minute scores had stabilized. (The median number of sessions required for the cry rate/minute to stabilize was 4 for each treatment-order group.) Comparisons were made between the final session of the first treatment and the second session of the second treatment. There was no difference in means between the infants exposed first to noncontingent stimulus provision and second to contingent stimulation, and the infants receiving the reverse order. When the two treatment-order groups are pooled, the Contingent mean of 1.95 cries/minute is found reliably greater than the Noncontingent mean of 0.06 cries/minute (t-test, $p < .01$). Therefore, maternal responding contingent on infant cries increased the rate of that response class relative to noncontingent maternal responding, in keeping with the expectation under instrumental-learning theory.

On the basis of this result, a tentative recommendation might be made to parents and other caregivers: If they are confident that they could discriminate physical-distress crying from other crying, they might attempt to respond to the distress crying but not to other crying of their infant.

Publications:

Gewirtz, J. L. Maternal responding and the conditioning of infant crying: Directions of influence within the attachment-acquisition process. In Etzel, B. C., LeBlanc, J. M., & Baer, D. M. (Eds.), New Developments in Behavioral Research: Theory, Method, and Application. Hillsdale, N.J.: L. Erlbaum Associates, 1977. Pp. 31-57.

Publications (continued):

Gewirtz, J. L., & Boyd, E. F. Does maternal responding imply reduced infant crying?: A critique of the 1972 Bell and Ainsworth report. Child Development, in press.

Gewirtz, J. L., & Boyd, E. F. In reply to the rejoinder to our critique of the 1972 Bell and Ainsworth report. Child Development, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00252-02 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Effects of Cognitive Impulsivity on Children's Academic Achievement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David E. Barrett Staff Fellow LDP NIMH

COOPERATING UNITS (if any)

Rowland Unified School District

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.45	.25	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To what extent do differences in cognitive tempo (reflection-impulsivity) identified in grade 4 account for variation in later academic achievement, independently of more general ability measures? Seventy children were administered the Matching Familiar Figures Test (MFF) in grade 4 and the Comprehensive Test of Basic Skills (CTBS) in grades 4, 5, and 6. Children identified as reflective based on performance on the MFF in grade 4 scored significantly higher on the CTBS achievement battery at all grade levels than those classified as impulsive. However, the two groups did not differ on achievement measures in grades 5 or 6 when scores were adjusted for initial differences in grade 4 CTBS. Although MFF error score and MFF response latency were each significantly predictive of grade 5 and grade 6 CTBS scores, neither of the MFF variables significantly improved the prediction of academic achievement when current level of achievement was controlled. Relations between reflection-impulsivity and academic achievement were stronger for middle SES children than for lower SES children.

Project Description:

Much attention has been given to the study of individual differences in "reflection-impulsivity," defined as the extent to which one reflects upon alternative responses when attempting to solve a problem with several available answers but high response uncertainty. Early studies in reflection-impulsivity indicated that children classified as impulsive, based upon low response latencies and high error scores on the Matching Familiar Figures Test (MFF), performed more poorly than those classified as reflective when tested in skill areas presumably related to academic success. Largely as a result of these initial reports, later research efforts have focused on possible psychological antecedents of cognitive impulsivity, personality characteristics of impulsive and reflective children, and the modification of cognitive tempo.

Although research interest in reflection-impulsivity stems largely from its apparent relation to children's academic performance, in no study has reflection-impulsivity been examined in relation to children's later academic achievement, as measured by standardized achievement measures. The present study is addressed to the question of whether cognitive tempo (impulsivity-reflectivity) is a significant and useful predictor of academic achievement in elementary school children.

A criticism of previous research on the correlates of reflection-impulsivity is that investigators have frequently failed to control for individual differences in level of cognitive development. To the extent that differences in MFF performance are a function of cognitive developmental level, interpretation of empirical relations between reflection-impulsivity and other dimensions of the child's cognitive functioning becomes problematic. The practical implications of this issue are equally important. If information about reflection-impulsivity does not improve one's ability to predict academic achievement beyond a level which can be achieved on the basis of more general ability measures, its usefulness with respect to educational decisions would have to be questioned. The present research, in examining relations between children's MFF performance and their later academic achievement, controlled for individual differences in current level of academic achievement. In this way, it sought to assess the practical as well as theoretical importance of MFF performance in the prediction of academic achievement.

Subjects were 95 children, grades 5 and 6. Children were administered the Matching Familiar Figures Test, the standard test for cognitive tempo, when in the fourth grade. The Comprehensive Test of Basic Skills (CTBS), a test of reading, language and mathematics, was administered when the children were in grades four, five and six. The CTBS correlates +.85 with total IQ on the Short Form Test of Academic Aptitude. Thus, it provides a measure of general intelligence as well as academic achievement.

The results of the present study show that while, in general, differences in reflection-impulsivity identified in grade 4 are predictive of individual differences in later academic achievement, a child's tendency toward impulsivity or reflectiveness has only negligible implications for later achieve-

ment when current ability level is taken into account. For both grade 5 and grade 6 achievement, the proportion of the variance attributable to differences in reflection-impulsivity (or to response latency and error score) is small in relation to the variance attributable to grade 4 achievement. Moreover, the increase in predictive power attained when one uses data from the MFF in addition to grade 4 achievement test scores to predict later academic achievement is generally nonsignificant.

One finding of interest was that for the lower social class children, not only did reflectives and impulsives fail to differ in grade 5 or grade 6 academic achievement when grade 4 achievement was controlled, they failed to show differences in the grade 5 and 6 achievement measures even when grade 4 CTBS was not included as a covariate. These findings suggest that a tendency toward impulsivity or reflectivity may have different behavioral implications among lower class children than among middle class children. While there is evidence that impulsivity is associated with fearfulness, inhibition, and anxiety about task competence among middle class children, we have little information about the personality characteristics of impulsive and reflective children within lower class populations. It has been suggested that the lower class child enters the standard testing situation with a different set of expectations, attitudes, and values than the middle class child. If this is so, the significance to be attached to an impulsive or reflective response style may differ as well. This is a final report.

Publications:

Barrett, D. E. Reflection-impulsivity as a predictor of children's academic achievement. Child Devel. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00253-02 LDP

PERIOD COVERED
July, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Behavioral Studies of Seriously Ill Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. J. Susman	Visiting Fellow	LDP NIMH
Other:	A. Hollenbeck	Staff Fellow	LDP NIMH
	S. P. Hersh	Psychiatric Consultant to Pediatric Oncology Branch	NCI
	A. S. Levine	Chief, Pediatric Oncology Branch	NCI

COOPERATING UNITS (if any)
Pediatric Oncology Branch, National Cancer Institute

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	1.00	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physical wellbeing is an assumed, though generally unmeasured, condition in studies of the child's psychological development and behavior. There is little developmental inquiry concerning the effects of loss or absence of physical well being on child development, despite the reality that acute or chronic ill health from disease or accident is, to a great or small degree, part of the life of every child. This project investigates the psychological and social impact of serious illness (cancer) on children and those in their social network (namely their families). Areas of research include coping abilities, the influence of the social system of the child and the mutual influences of the stressed children and those of their social network. Observations, interviews, and parent and nurse ratings are being employed to investigate these phenomena.

Project Description:

The overall purpose of this project is to assess the impact of a serious illness (cancer) on psychological processes in children, with particular emphasis on the following dimensions: (1) the child's understanding of the illness, (2) the child's subjective experiences associated with illness, and (3) the child's social behavior with significant others.

In recent years, the study of death and dying has gained respectability as a scientific issue. The dying child's awareness of impending death has been the subject of an increasing number of research efforts. However, advances in medical treatment have increased the probability of prolonging life and mortality rates have declined for a number of illnesses. Within this context, it is of interest to focus on a child's experience with the process of the illness rather than the result. The current study focuses on the problem of coping with a serious illness.

The integrity of the body is an important developmental issue for children in good health. Anxieties over imagined or feared bodily injury and anxieties over physical adequacy contribute considerably toward shaping the child's self concept, psychological defenses, body image, and overall quality of life. Since concerns about the body are a psychological issue for healthy children, it follows that children suddenly faced with the loss of body integrity and with the very real threat to life experience great psychological stress.

The sample chosen for study was a group of children with metastatic pediatric solid tumors and lymphomas which had failed to respond to more conventional therapeutic regimes. These children receive highly experimental intensive chemotherapy. Such therapy is itself very toxic and can lead to life-threatenin or fatal complications. Research participants are randomly assigned to Laminar Air Flow units (experimental group) or the general pediatric oncology ward (control group) for a period of five-to-seven weeks while they are receiving chemotherapy. The purpose of Laminar Air Flow rooms is to provide a protective germ free environment. Because the patient's biological defenses are especially compromised by marrow failure, immunodeficiency, or a malignancy during this period of chemotherapy, it is thought that remaining in a Laminar Air Flow room may help to ward off endogenous and exogenous sources of infection. The research participants include patients and their families on the Pediatric Branch of the National Cancer Institute.

Study A. Changes in conception of illness and social behavior of children in protected environments

Research participants include children 9 to 21 years, randomly assigned to Laminar Flow or general pediatric oncology. In Laminar Flow units, these children are deprived of important social contacts--parent, siblings, and peers--as well as the normal sources of stimulation in the environment. Body integrity is threatened by weight loss, alopecia, and painful medical procedures associated with chemotherapeutic agents. Although pain and isolation are extreme for these children, the conditions are not unique to illness and are of considerable significance in children's experience.

The research questions are designed to explore the child's understanding of the illness and treatment and to identify attributes of the medical environment that are stress provoking or anxiety reducing. Major dimensions of the research questions include: (a) What is the child's and parent's conception of the illness--its cause, course, and healing process--and how does this change over time? (b) What is the relation between their conceptions of the illness and their emotional states and social behavior during hospitalization? (c) What are the child's concerns about death and when are these concerns manifested? and (d) What role do parents and medical personnel play in providing comfort and support to the distressed child?

Multiple methods are used for data collection: systematic observation; interviews; and self, parent, and nurse ratings. Because the research issues are questions of change and interaction, regular and extensive sampling on a continuing basis throughout the period of hospitalization is necessary to assess sequences and interdependencies of ongoing events and psychological processes. To date, data have been collected on six research participants; four adolescents confined to Laminar Flow and two controls. Ten more children will be studied over the next five months. Data are being analyzed for both intra- and interindividual change. Behavioral data will be analyzed in conjunction with physiological measures of clinical status.

Early impressions support the conclusion that children and parents have an accurate and sophisticated knowledge of the particular disease and treatment protocol. In some instances, children adopt a particular causal model to explain their illness. For instance, osteogenic sarcoma patients believe that long-bone trauma is the causal agent. Prior to chemotherapy, death is sometimes mentioned as a possible outcome if medical interventions were not available. Future orientation is not realistic, with an inordinate degree of hope being placed on the remission-inducing-potential of chemotherapy. In the initial phase of chemotherapy, mother and nurse rate the child as happy, outgoing, and relaxed. As chemotherapy itself alters physical well-being, hope for the future declines for the child. The loss of physical well-being is associated with an increase in negative affects and concern with death. Discussion about death is usually done indirectly. The child may discuss the death of others on the unit and compares the others' physiological status to his/her own status. The child rationalizes his own existence by denying that (s)he is in a similar deteriorating state. The child distinguishes between being sick and having a disease--(s)he is not sick because of the disease itself, but rather sick from the side effects of the treatment. The child's thoughts and fears are mainly concerned with the technical aspects of treatment: intravenous infusions, drugs, and hematology results. After chemotherapy, hope for the future is pessimistic compared to before treatment. Time and future are viewed within the framework of the child's schedule of maintenance chemotherapy (five days in the hospital out of every 17 days). Children express an ambivalence about having gone through the treatment or returning to their home community. These self reports are

corroborated by parent ratings of the child's moods. Observational data reveal that children predictably withdraw from interactions with others during the period of severe granulocytopenia. This period coincides with the period of greatest concern about death.

In summary, the children are not morbidly obsessed with death but are concerned with the task of living. Initial hope for cure permits denial of death and the fears associated with it; a truncated view of time shields them from considering the probable future consequences of their illnesses. Yet the loss of physical well-being, even when secondary to chemotherapy and the reality that maintenance chemotherapy is necessary even after their Herculean distress associated with the ablative chemotherapy, leads to recognition of the existential reality of death. The experience of physical illness for the child appears to bring about an assimilation of an abstract concept of death.

Data collection will continue for the next half year. Codings of data are carried out as the data is collected.

Study B. Behavioral observations of young children in reverse isolation deprived of maternal contact.

The deleterious effects of separation in childhood have been demonstrated in experimental studies with animals and in naturalistic observational studies of children. Harlow's work with infant monkeys underscores the necessity of physical contact comfort early on in life for the later development of normal social functioning. Social learning theorists (e.g., Walters and Parke; Rheingold) have shown the relevance of distance receptors (audition and vision) in forming attachment bonds. Recent evidence suggests the combined significance of close physical contact plus continuing reciprocal involvement between the mother's and the child's auditory and visual perceptual systems. Human infants appear to need close physical contact with another human--contact which contains meaningful auditory and visual stimulation.

Recent animal studies on monkey mother-infant separation have pointed to physiological changes occurring in the infant almost immediately after separation from the mother. Behavioral signs of clinical depression in the infant monkey emerge later. These effects appear to be most deleterious when visual contiguity is maintained.

The Laminar Air Flow situation provides a unique opportunity to study human mother-child separation in a naturally occurring situation that is analogous to experimental studies of mother-infant monkey separation. A 3-year-old girl and a 3½ year-old boy were each placed in Laminar Flow for a 6 week period while undergoing chemotherapy for malignant tumors. Specific research questions focus aroun7 mother-child separation during therapy. (1) How does

separation per se influence observable behaviors normally associated with childhood (levels of play, aggression, affect) (2) What are the physiological concomitants related to separation? (3) Are there cumulative psychological effects due to separation? (i.e., prolonged bouts of anxiety or depression).

The physical setting of the present study was identical to that described for Study A. However, data collection procedures differed due to the age of the children and the more focused research question on the effects of separation. Videotaped observations were collected at two points in the diurnal cycle, morning and afternoon, four times a week, during the period of the child's hospitalization. Subsequently, these observations were coded by two trained observers using a coding scheme that assessed dimensions of the child's role (initiator--receiver), behavior (play, sleep, protest, etc.), communications (talk, humor, praise), and affect (happiness, sadness, etc.). Physiological measures of heart rate, blood pressure, body temperature and respiration were collected four times daily.

Analyses are in progress. Positive behaviors such as play tend to decrease, and negative behaviors such as protests tend to increase over the period of separation. The analysis will focus on both qualitative changes and sequential changes in behavior related to the course of illness and treatment procedures. Particular attention will be given to symptoms of behavioral depression over time. For example, a sequence of increasing symptoms of depression might be described behaviorally as follows: The child's initial play and smiles may gradually be replaced by passive responses with no facial expression.

Preliminary results indicate that there are initial drops in body temperature following separation, in Laminar Flow. These changes appear to be independent of treatment and disease related fluctuations. While the pattern of physiological measures differ for the two children, both patterns indicate a general decline in metabolic rate. Videotaped observations are being analyzed for patterns of behavioral changes in relation to physiological data and clinical states.

These analyses will be completed in the next six months, and a report will be prepared.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00255-01 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Maternal Imprisonment: Psychological Influences on Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Velma LaPoint	Guest Worker	LDP, NIMH
	Marian Radke-Yarrow	Chief, LDP, NIMH	LDP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Among social conditions that may be assumed to place children at high risk for the development of mental health problems is the child's lack or loss of stable and supportive parenting. When the reasons for inadequate parenting are themselves indicative of psychological problems of the parents, risk for the child is compounded. This is a study of psychological consequences for children whose mothers are imprisoned, a situation placing children at risk for problems. Children are confronted with separation from mother who is defined as "bad" and needing punishment, disrupted living arrangements, and economic disadvantages. Relationships maintained during mother's incarceration, and children's affective and social behaviors are studied in 80 to 100 families. Selection of variables is based on findings regarding the effects of parent-child separations on children's development. Community living single-parented families, comparable racially (Black) and socio-economically, are also studied. Data are diary records, individual tests and observations of mother-mother, mother-child, and child-child interactions in controlled situations.

Project Description:

Among social conditions that may be assumed to place children at high risk for the development of mental health problems is the child's lack or loss of stable and supportive parenting. When the reasons for inadequate parenting are themselves indicative of psychological problems of the parents, risk for the child is compounded. Maternal imprisonment is often a marker for a set of circumstances, each of which, alone, presents potentially difficult conditions for children: The mothers are predominately of very low income families; most are single parents; they were often adolescents when their first pregnancies occurred; living conditions and employment histories are often unstable. In addition, in the events preceding and surrounding imprisonment and in the penal and correctional policies governing mother's communications, children are more than likely to experience stress.

The purpose of the study is to determine the psychological consequences for children of mother's imprisonment. Effects on children will be assessed in the domains (1) of children's emotional states, particularly feelings of anger, depression, fear, and confidence in self; (2) their relationship with adult authority figures, their mothers particularly, but also maternal substitutes and school and societal authorities; their peer relationships with regard to self isolation, conflict and cooperation; and their abilities to mobilize themselves to meet demands such as required for school achievement and problem solving.

Eighty to 100 imprisoned mothers and their children are being studied. Moth have been in prison for at least six months when they enter the study. Thei offenses include both violent and nonviolent crime. They are mainly first offenders; some have been in prison one other time. The children studied are between 5 and 14 years. Community-living single-parented families comparable racially and socio-economically, without histories of long term institutionalization, are also being studied. Data are in the form of diary-like records, individual test responses, and observational records of mother-mother, mother-child, and child-child interactions in controlled situations.

Pilot work on this project has included preliminary data gathering from 31 imprisoned mothers, at the Maryland Correctional Institution for Women, and their children. Contact with mothers consisted of group discussions. Each group consisted of three to five women. Tape recordings of group session were done with consent of participants. From these discussions we have learned much about this population, about their experiences as offenders, as imprisoned mothers, and indirectly, about their children's experiences. The children of participating mothers were permitted to visit their mothers for several hours. Semi-controlled group settings permitted observations of mothers and children. The procedures were modified several times in the course of the preliminary work to attempt to approximate the systematic procedures to be used in the next phase. Data collection with systematic procedures will be carried out over the next year.

From these preliminary data, several impressions emerge: Mother's imprisonment is denied and concealed in many ways. The processes resemble those reported by Yarrow et al. in families' treatment of mentally ill members. Children were warned and disciplined not to reveal the facts of mothers' status. The children are often exposed to illegal and violent actions by their mothers (including murder); in no case were there reported interventions to help the child cope with the stresses involved.

Publications:

None

INTERAGENCY SCIENCE INFORMATION EXCHANGE (PROJECT NUMBER (DO NOT use this space))	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00256-02 LDP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Expressions of Affect: Children's Age and Sex-role Expectations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.A.L. Pizzigati	Staff Fellow	LDP NIMH
OTHER:	C.F. Halverson, Jr.	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Green Acres School, Rockville, Maryland

Laboratory of Developmental Psychology

DEPTH

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.00	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

While assumptions about appropriate sex-role behaviors are culturally acknowledged, little is known about the development of differential perceptions of sex-role personality dimensions. Children's and teachers' judgments with regard to sex-role-related personality traits are investigated from a developmental perspective. Children, ages 5-14 years, viewed videotaped skits portraying traits stereotypically associated with one sex or the other (e.g., gentleness, stoicism). The children evaluated themselves, considered peers in terms of the traits portrayed, and assessed the social desirability of the traits. Teachers rated the children on the traits and on aspects of social ease (e.g., nervousness). Preliminary findings indicate that although there is a subsample of children who evaluated themselves stereotypically, for the total sample, girls rated themselves higher on some feminine traits than did boys, but there were no sex differences for the masculine traits. Trait conceptualization, self-evaluation, and the ability to disregard social desirability in making self evaluations were related to age for some of the traits.

Project Description:

The purpose of this project is to investigate the development of children's perceptions with regard to sex-role-related personality traits. The importance of the gender dimension in children's perceptions of themselves and their peers was studied. Also, teacher ratings of the children on the personality traits were compared with the children's evaluations of themselves. The traits selected for study are gentleness, fickleness, emotional expressiveness, dependency, nurturance, stoicism, messiness, assertiveness, independence, and boastfulness. Evidence from the literature and from pilot data suggest that these traits relate to sex-role attitudes.

The sample consists of 150 middle and upper-middle class elementary school boys and girls attending a private school. Each participant individually viewed 10 videotapes portraying the traits listed above. For each trait, the children were asked to describe the portrayal, evaluate themselves in terms of the trait portrayed, nominate two peers who exhibit the trait, and evaluate the social desirability of the trait.

Data collection has been completed. Preliminary findings indicate that althoug there is a subsample of children who evaluated themselves stereotypically, for the total sample, girls rated themselves higher on some feminine traits than did boys, but there were no sex differences for the masculine traits. Also, there were developmental differences in the children's manner of trait conceptualization and self evaluation for some of the traits, with young children exhibiting more concrete style than the older children. The younger children also showed more dependency on social desirability when evaluating themselves than did the older children.

This investigation will help to elucidate the course of sex-role development and some of the socialization factors influencing children's orientations to those around them.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00257-01 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.A. Moss	Research Psychologist	LDP NIMH
OTHER:	D.G. Poplack	Senior Investigator Pediatric Oncology Branch	NCI
	P. Pizzo	Senior Investigator Pediatric Oncology Branch	NCI
	N.P. Ramu	Clinical Associate Pediatric Oncology Branch	NCI
	S. Siegel	Chief, Div. of Hematology-Oncology	Los Angeles Children's Hospital, Los Angeles, Calif.

COOPERATING UNITS (if any)
NCI
Los Angeles Children's Hospital

Laboratory of Developmental Psychology
DEPT.

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.90	.70	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of CNS treatment (cranial irradiation and intrathecal chemotherapy) on the cognitive and perceptual functioning of children with leukemia are investigated. One study compares a group of leukemic children in remission with a matched group of sibs on measures from a battery of psychological tests (Wechsler, Bender-Gestalt and Graham-Kendall). Computerized tomographic scans on the patients as a measure of change in brain structure will also be related to their intellective functioning. A second study deals with another sample studied prospectively. In this instance children in remission from leukemia are evaluated on the same battery of psychological tests as in the first study. They are tested just prior to prophylactic CNS treatment and are re-evaluated one year later to determine if any possible brain changes brought on by the CNS treatment have resulted in learning disabilities and diminished intellectual functioning. A normal control group will be tested at both points in time in order to check for testing effects. The composition of this sample will allow for evaluating the effects of age and variation in treatment as contributing factors toward deleterious outcomes.

Project Description:

The chance of survival from childhood leukemia has increased greatly over the past 10 years because of the utilization of new treatment procedures. Whereas prior to this time, there was minimal likelihood of any enduring remission, now, approximately 50% of the children treated for leukemia remain in remission for longer than three years after the initiation of treatment. The main change in treatment that has led to this improved outlook is the inclusion of prophylactic measures consisting of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents. These measures are aimed at destroying any stray leukemic cells in the brain and central nervous system.

There is little evidence as to the possible deleterious effects on the brain of this central nervous system (CNS) treatment. In the few studies that have been done, results are at best suggestive and equivocal. The studies tend to be based on very small samples, and lack adequate controls.

The purpose of the proposed research is to determine if CNS treatment has a deleterious effect on intellectual functioning and to evaluate the nature and extent of these effects. An additional objective is to investigate whether any identifiable brain pathology associated with CNS treatment is related to any particular pattern of intellectual deficit. Two kinds of assessment are involved: (1) inspection of photographic x-rays of the brain (computerized tomography, or C-T scans), and (2) psychological testing.

This research is conducted in collaboration with the Pediatric Oncology service of the National Cancer Institute (NCI) and the Division of Hematology-Oncology of Los Angeles Children's Hospital. Two studies are in progress. One involves evaluation of intellectual functioning only after CNS treatment (post-treatment study) and the other study consists of comparing intellectual performance prior to and subsequent to CNS treatment (prospective study).

Because of the age range of our subjects the three Wechsler Intelligence Tests are used: WPPSI (ages 2½ - 6), WISC-R (ages 6 - 16), WAIS (over 16 years of age).

Study I. Post-treatment study

The sample consists of 36 children who have been in remission for 2 to 5½ years from acute lymphocytic leukemia (ALL). Some of these children are still on chemotherapy since this treatment continues for three years after remission. The mean age of the children at the time of assessment was 11.5 with a range of 4 to 21 years of age. This study also included a control group of 34 of the siblings of children in the patient group. The siblings selected for the control group were of similar age to the patients (a mean age of 11.7 with a range of 4 to 23 years of age). All children were administered a Wechsler Intelligence Test, the Graham-Kendall and the Bender-Gestalt. This battery of tests will be readministered to both groups approximately one year after the initial testing. Readministration of the tests to the control group

provides an estimate of test practice effect.

The main analysis consists of comparisons between the patient and the control group. Although all of the patients received CNS irradiation, intrathecal medication used with half the subjects was methotrexate, and with the other half, arabinoside. Analyses, therefore, include evaluation for possible differential effects from these two drugs.

Intellectual functioning will be assessed also in relation to C-T scans, which provide measures of deviancy in brain structure. It is expected that brain damage will have less effect on already acquired skills and knowledge then on the capacity to learn and acquire new skills. Since the time interval between CNS treatment and the first psychological evaluation varies, time since treatment will be examined in relation to degree of intellectual deficit. Follow-up testing one year after the initial testing will provide additional information on possible brain damage. Information about school history, obtained from the mothers, will be used to determine if alternative explanations, other than brain damage, account for decreased intellectual efficiency.

The initial psychological testing of the patient and control groups has been completed. Some preliminary comparisons between these groups indicate that the patient group has a lower overall IQ ($z = 3.78$, $p < .0001$ level, one tailed test) and greater discrepancy between Verbal and Performance IQ ($z = 2.09$, $p < .02$ level, one tailed test) than their sibling controls. The mean IQ's were 99.3 for the patients and 111.8 for the siblings. The mean difference (discrepancy) between Verbal and Performance IQ's was 10.3 for the patients and 7.5 for the sibling controls.

Study II. Prospective study

A sample of 60 children with acute lymphocytic leukemia, who receive prophylactic CNS treatment, will be administered the same psychological evaluation described in the Post-treatment study. This assessment will be carried out before CNS treatment and one year after treatment. This interval of one year between baseline and follow-up testing should be sufficient to allow for loss in learning abilities if such losses occur as the result of treatment. The sibling group from the Post-treatment study will be used as a control group for this study to partial out any practice effects that may contribute to the follow-up IQ scores among the patients.

Half of this sample will be between 3½ and 6 years of age and will be administered the WPPSI, and the other half of the sample will be between 6 and 16 years of age and will be administered the WISC. The age distribution for this sample will provide a good basis for evaluating whether age at the time of CNS treatment is a factor in contributing to the degree of intellectual deficit that may occur.

A small separate group of children with optimal prognosis (between 4 and 7 years of age and with a white cell count of less than 10,000) also will be studied prospectively. Because of their unusually good prognosis, half of this group will receive intrathecal methotrexate without the CNS irradiation while the other half of this group will receive the complete CNS treatment. This sample will allow us to evaluate the effects of CNS methotrexite without irradiation.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00258-01 LDP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Relations Between Minor Physical Anomalies and Neonatal Sex Hormones in Normal Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.F. Halverson, Jr.	Research Psychologist	LDP NIMH
OTHER:	E.E. Maccoby	Professor	Stanford Univ. Stanford, Calif.
	C.N. Jacklin	Sr. Research Assoc.	Stanford Univ. Stanford, Calif.

COOPERATING UNITS (if any)

Department of Psychology, Stanford University, Stanford, Calif.

LAB / BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An index of minor physical anomalies will be related to individual differences in the level of androgen and estrogen circulating in cord blood at birth. Previous research has identified both androgenic hormones and the incidence of minor physical anomalies as related to active, aggressive behaviors in young children. In the Stanford Longitudinal Study, under the direction of Drs. Eleanor Maccoby and Carol Jacklin, the neonatal hormonal assays for this group of children have been collected. As part of the follow-up of these children at age 18 months, the presence of minor physical anomalies have been assessed. Analyses will be conducted to see if the anomaly index is related to the level of sex hormones present at birth. If anomalies index the level of masculinizing testosterone present at birth, an important antecedent of the incidence of multiple minor physical anomalies as well as the mechanism which would explain the relations found previously between active, aggressive behaviors and anomalies will have been identified.

Project Description:

A number of investigations carried out in this Laboratory have been concerned with the biological and environmental contributors to the expression of high activity behavior in young children. These studies have demonstrated a relation between the presence of minor physical anomalies at birth and impulsive and aggressive behavior in boys aged 2 years to 12 years. Eighteen anomalies were used which can be assessed at any age and consist of minor growth abnormalities of the head, hands, and feet, such as epicanthus, hypertelorism, low set ears, high steepled palate, single palmar creases, partial syndactyly of the toes, etc. These anomalies are considered the cardinal stigmata of trisomy 21 and have been shown to occur in normal samples. Based on more detailed behavioral data at age three, it has been found that the incidence of anomalies at birth predicts the behavioral triad of impulsivity, short attention span, and high activity for 3-year-old boys. It has generally been thought that anomalies are possible sequelae of noxious events which occurred during the development of the embryo.

There is another line of research relevant to the present study which focuses on high activity behaviors which differentiate boys from girls. The hypothesis is advanced that differences between the sexes in the triad of aggression, high activity, and impulsivity, may be sequelae of differences in the amount of fetal androgen present for the differentiation of the male fetus. In studies of primates, the administration of testosterone propionate (TP) prenatally and postnatally has been shown to increase levels of aggression, high activity, and "rough-and-tumble" play. In light of these two converging lines of research, the hypothesis of the present research is that both minor physical anomalies and fetal testosterone are related to the behavioral syndrome characterized as "male behavior," and further that the index of minor physical anomalies is related to the amount of fetal androgens present during sexual differentiation of the fetus. It seems possible that, for some males, the minor physical anomalies index could be a possible marker for male differentiation. That is, the more anomalies, the more virilized the male infant and the greater the likelihood that he will subsequently show high activity, aggressivity, and impulsivity.

The present research is being carried out in collaboration with Drs. Eleanor Maccoby and Carol Jacklin (Stanford University), who are conducting a longitudinal study of behavioral development. They have obtained hormonal assays of maternal cord blood on a sample of approximately 70 normal, full-term infants. These children have now reached 18 months of age and have been assessed for the presence of the 18 minor physical anomalies. The objective of the project will be to assess the relation between the anomaly index and neonatal hormone levels and further to analyze their joint contribution to temperamental and aggressive behavior. Data collection will be completed in September 1977. This is a final report. The investigator is leaving the institution and will continue his work in his new position.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00259-01 LDP

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)
Relations between Parent, Teacher, and Clinician Reports of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Achenbach	Research Psychologist	LDP NIMH
Other:	C. Edelbrock	Staff Fellow	LDP NIMH
	F. Dickerson	Clinical Psychologist	Woodburn Center for Community Mental Health, Annandale, Va.

COOPERATING UNITS (if any)
Woodburn Center for Community Mental Health, Annandale, Va.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION
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INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
20	.10	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relationships between parent, teacher, and clinician reports of the behavioral problems and competencies of children referred for outpatient mental health services; (2) to determine the relationship between children's presenting behavioral patterns as reported by these sources and the outcome of their clinic contacts. At intake and again following termination with the clinic, parents and a clinician fill out the Child Behavior Checklist, while the child's teacher fills out a Teacher report form. Child Behavior Profiles scored from these data will be compared to identify agreements and disagreements between parents, teachers, and the clinician. The relationship of outcome to intake Profile patterns, demographic variables, and other characteristics of the children will be assessed in order to identify predictors of poor outcome. If children who typically have poor outcomes can be identified, new treatment strategies will be developed to help them.

PHS-6040
(Rev. 10-76)

Project Description:

Little is known about the relationships between client characteristics and the effectiveness of existing treatments for child psychopathology. One of the purposes of the Child Behavior Profile (Project No. Z01 MH 00244-02LDP) is to differentiate among children who differ in their responses to various treatment modalities. This study is designed to determine the degree of agreement and the nature of disagreements between Profiles scored from Child Behavior Checklists completed by children's parents, their teachers, and a clinician who does an intake and follow-up evaluation but is not involved in treatment. It is also designed to determine whether classification of children according to Profile patterns derived from reports by each type of informant separately and/or in composite is predictive of outcome in response to the child mental health services offered by a community mental health center.

In addition to the Behavior Profiles, the intake clinician will obtain data on family composition, demographic characteristics, previous mental health contacts, degree of parent motivation for help, and the problems considered most serious by the parents. One month after the family's termination with the clinic, the intake clinician will contact the family to obtain Behavior Checklists describing the child's current status, plus data on changes in the problems considered most important by the parents, relevant changes in the family, the family's reactions to the services they have received, and possible needs for further services.

If children who have poor outcomes can be discriminated from those who have good outcomes on the basis of intake data, then new treatment strategies can be developed for helping children who are not likely to benefit from mental health services as currently offered. The intake data collection begins in September 1977 and continues for one year. Continuation beyond that time will depend upon the findings obtained up to that point.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00260-01 LDP

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)
Intellective Development in Normal and Deviant Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. J. Caron Research Psychologist LDP NIMH

Other: Mary Coleman Director Children's Brain Research Clinic

Rose F. Caron Adj. Prof., Dept. of Psychology, Geo. Wash. Univ.

COOPERATING UNITS (if any)
Children's Brain Research Clinic; George Washington University

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.20	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present project will compare normal and deviant infant samples in terms of their ability to abstract relational features of both the physical and social environment. It is assumed that intellective deficiency is best reflected in these functions and hence that they may provide the basis for early diagnosis of mental handicap. A variety of tasks requiring discrimination of invariant configural and temporal-causal properties will be administered to normal, Down's syndrome, and "high risk" infants at various gestational and postnatal ages during the first year of life.

PHS-6040
(Rev. 10-76)

Project Description:

Despite the acknowledged importance of early infancy in the development of adult mentality, little is presently known about the basic intellective processes (attention, coding, memory, discrimination, abstraction) by which normal infants extract and assimilate information from their surround. In the absence of solid data on such questions as the age-related limits of these capacities, the course of their development, or the external conditions implicated in their ontogenesis, we are hard put either to identify intellective disorder in infancy or to remediate suspected deficit and optimize growth. This is particularly unsettling, given that thousands of newborns are discharged annually from hospitals as being at "high risk" for later mental and neurological handicap.

To facilitate detection of early intellective aberration as well as to gain further leverage on the early stages of normal cognitive development, the present project will compare normal with deviant infant samples on recently developed experimental tasks which are much more reflective of higher-order information processing functions than standardly employed infant psychometric scales. Down's syndrome infants (mongoloids), whose chronic genetic anomaly clearly predisposes them to future retardation (most adult IQ's range between 25 and 45), will be compared with normal infants. Since the primary sensory systems of these infants are relatively intact, any deviations from the normal range can reasonably be attributed to deficiencies at higher levels of nervous functioning. Therefore, comparison of Down's and normal babies provides a unique strategy for differentiating those aspects of normal development which are mediated by central mechanisms. Furthermore, the same behavioral measures which yield differences between the Down's and normal child can be used to assess intellectual deficit in infants at risk because of other factors (preterm babies or those exposed to varieties of prenatal or perinatal insult).

The working assumption of the present project is that a critical factor in emerging intelligence is the ability to process relationships. Early perceptual development has been characterized as a progression from the detection of isolated features to the differentiation of invariant structure (i.e., features in relationship) and ultimately to the abstraction of inter-relations between structured objects (cf., E. Gibson, 1969). Likewise, current theories of meaning stress that things (words or objects) are never comprehensible in isolation but only in relation to other things. Competence in infancy, then, may be intricately related to the proclivity to detect invariant relationships, which in turn may be an important predictor of later intellectual achievement.

The research focus, consequently, will be on the sensitivity of normal and defective infants to various spatial and temporal relationships. The emergence of the ability to detect relationships will be studied primarily by means of the visual habituation paradigm. To illustrate, infants will be habituated to a target containing a number of configured elements, and,

once habituated, will be tested for dishabituation to a new arrangement of the same elements. Alternatively, the elements, rather than the configuration, will be changed following habituation. Generally, we anticipate that normal infants will be more responsive to relational change than defective infants of comparable age, and that the latter will be more attentive to element change than normals. Preterm infants will be compared to normals both in terms of conceptional age and postnatal age to test the relative contributions of maturation and experience to the development of these cognitive processes.

A variant of the preceding paradigm will test the infant's ability to abstract an invariant configuration across changing elements. On each habituation trial the same configuration will be comprised of different components. Infants who are attuned to the configuration should habituate more rapidly than those who are not so attuned. They should also show stronger recovery (dishabituation) to new elements arranged in a different configuration than to new elements in the same configuration, whereas non-relationally sensitive infants should yield comparable recovery to both targets.

Relational abstraction will be tested not only with physical stimuli but also with social stimuli. For example, sensitivity to a facial expression (e.g., a smiling face) could be examined by displaying a different smiling face on each habituation trial followed at test by a new face, both smiling and frowning. Temporal-causal relationships will also be investigated by habituating infants to event sequences (either live or on film), which again may be either physical or social. The subject could be habituated to a sequence in which A pushes B, and, following habituation, might be shown A hugging B, B pushing A, or C pushing D. Alternatively, the habituation trials might consist of A pushing B, C pushing D, E pushing F, etc. and the recovery trials, of X pushing Y vs. X hugging Y.

Tasks which yield the greatest differences between Down's syndrome and normal infants will be used to assess intellective deficiency in infants at risk.

Piloting of stimulus material is presently underway.

Publications:

None.

INTRAMURAL SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00261-01 LDP

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Accuracy of Hierarchical Clustering Solutions in Multinormal Mixture Analyses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Craig Edelbrock, Ph.D. Staff Fellow LDP

COOPERATIVE UNITS (if any)

LAB

Laboratory of Developmental Psychology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.30	.30	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) A major statistical problem in mental health research has been how to translate subjects' scores on a set of single dimensions into a useful classification of individuals. This problem is particularly significant since decisions (e.g., regarding treatment assignment) must be made about whole individuals, rather than scores on single dimensions. The purpose of this research was to supply empirical criteria for our and other's use when choosing an appropriate clustering algorithm. Parametric comparisons were made between hierarchical clustering algorithms on their ability to retrieve the underlying populations from multinormal mixtures. Accuracy was defined as the agreement between the obtained clusters and the underlying populations, and was measured by the statistic kappa . Algorithms using the product-moment correlation coefficient as a measure of similarity were superior to those using Euclidean distance. At high levels of coverage, accuracies were higher for standardized vs. unstandardized data sets. Average linkage using correlation had the highest accuracy values, followed by Ward's error sum of squares technique and centroid linkage (weighted pair group average linkage) using correlation. Complete linkage and single linkage algorithms were significantly less accurate than these algorithms, regardless of the measure of similarity used.

Project Description:

A major statistical problem in mental health research has been how to translate subjects' scores on a set of single dimensions into a useful classification of individuals. This problem is particularly significant since decisions regarding diagnosis, treatment assignment, and delivery of services must be made about whole individuals, rather than their scores on rating scal symptom checklists, or questionnaires. Cluster analysis is a family of statistical procedures that is becoming more commonly used in mental health research to produce classifications of individuals from a multivariate data set. Unfortunately, many clustering algorithms are available, different algorithms produce different results, and some algorithms are clearly unsuited for the type of data used in behavioral research. Hence, it is difficult to choose an algorithm for a clustering application, and to place confidence in the resulting typology.

This research compared several of the most widely used hierarchical clustering algorithms on their ability to retrieve the underlying data sets from multinormal mixtures. The purpose of this research was to supply empirical criteria for our and other's use of cluster analysis for choosing a clustering algorithm and measure of similarity, and for deciding whether to standardize data before clustering.

Five amalgamation rules were analyzed including (1) single or minimum linkage, (2) complete or maximum linkage, (3) average linkage (unweighted pairwise group mean average linkage), (4) centroid linkage (weighted pairwise linkage), and (5) Ward's error sum of squares technique. The first four algorithms used either the product-moment correlation coefficient or Euclidean distance as a measure of similarity, yielding nine algorithms altogether.

The data sets were 10 multinormal mixtures, generated using monte carlo techniques. The number of underlying populations in each mixture ranged from 2 to 5, and the number of variables ranged from 7 to 22. Means for the variables were sampled from the range of 40 to 60, with a measurement error of $\pm$ .60 of the standard deviation of the variable. In addition, the ten data sets were prepared in either standardized or unstandardized form, resulting in a total of 20 data sets.

Accuracy of the clustering solutions was defined as the agreement between the obtained clusters and their optimally matched underlying populations. This agreement was measured by the statistic kappa, given by the formula:

$$k = \frac{P_o - P_e}{1 - P_e}$$

where P_o is the proportion of observed agreements between the clusters and the underlying populations, and P_e is the number of agreements expected by chance. Accuracies could range from 1.00, indicating perfect agreement to 0.00, indicating no more than chance level of agreement.

For each hierarchical tree produced, accuracy was calculated at 100% coverage, where the number of clusters equaled the number of underlying groups, and at successively lower levels until k = 1.00. In order to compare accuracies across algorithms and data sets that did not produce equal levels of coverage, linear regression was used to interpolate between empirically derived points and estimate 7 common levels of accuracy including 60%, 70%, 80%, 90%, 95%, 99%, and 100% coverage.

A 4 x 2 x 2 (amalgamation rule x measure of similarity x standardization) repeated measures ANOVA was used to compare the accuracies of the first four algorithms. Statistical comparisons with Ward's technique were made using the paired-t test. Several effects in the ANOVA were significant including the effect of the amalgamation rule, the measure of similarity, the amalgamation rule x measure of similarity interaction, and the repeated measures x standardization interaction.

Overall, the algorithms fell into two groups with regard to relative accuracy. The group having relatively high accuracy included average linkage, centroid linkage and Ward's technique, and the group with relative low accuracy included single and complete linkage. Algorithms using the product-moment correlation were superior in accuracy to those using Euclidean distance. In addition, at high level of coverage, accuracies were higher for standardized average linkage using correlation, followed by Ward's technique and centroid linkage using correlation--the latter two being not significantly different from each other. Complete and Single linkage algorithms were consistently less accurate than these three algorithms, regardless of the measure of similarity used. This is a final report.

A manuscript summarizing this research is in preparation.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00262-01

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Acquisition of Sex-Typed Preferences in Young Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Craig Edelbrock, Ph.D. LDP/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.70	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The investigator has developed a measure of sex role acquisition for young children that assesses the degree to which the child's preferences for various activities and roles adhere to either cultural sex role stereotypes or the child's own conceptions of what is sex-appropriate behavior. The sex and age of the child, cognitive level, and the sex of the experimenter were found to affect children's sex role preference scores, and boys and girls differed in their application of sex role standards to adult vs. child behavior. Girls adhered more to their own conceptions of what was sex-appropriate than to sex role stereotypes when considering children's play activities, but adhered to the stereotyped feminine role when considering adult activities and roles.

Project Description:

The Sex Role Learning Index (SERLI) is a picture choice instrument developed to compare young children's preferential choices of various play activities and adult roles to both cultural sex role stereotypes and each child's conceptions of what is sex-appropriate. The SERLI consists of 60 black-and-white line drawings depicting child play activities and adult roles, as well as the objects associated with those activites and roles. In administering the SERLI, the child is first asked to classify the object items into the categories "for boys," "for girls," and "for both boys and girls." The child is then asked to choose from two sets of items, one depicting Child Figures (CF) and the other depicting Adult Figures (AF), those items the child would like best to do, play with,or be. Hence, in each section (CF and AF), the child's choices can be scored relative to either the sex role stereotypes of the items, or the child's own conceptions of which items were sex-appropriate as revealed in the classification of the objects.

To assess the adequacy of the SERLI, 119 preschool aged children were given the SERLI, the It Scale for Children, and the Peabody Picture Vocabulary Test. Boys showed higher masculine preferences in the AF than CF section, while girls had higher feminine scores in the AF than CF section. Boys were more masculine than girls were feminine in the CF section, but not in the AF section. Girls were also found to adhere more to their own conceptions of what is sex-appropriate than to sex role stereotypes, in the CF section.

Boys' scores in the AF section correlated significantly with mental age ($p<.05$) and the IQ ($p<.01$), while girls scores in the CF section correlated significantly with chronological age ($p<.01$). Boys scores on the SERLI were also significantly correlated with scores on the It Scale for Children ($p<.05$), but girls' scores were not. Three week test-retest reliabilities on the SERLI were higher for children tested by a same-sexed experimenter than for those tested by an opposite-sexed experimenter. This is a final report.

A manuscript summarizing this research has been submitted for publication.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00263-01

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)
Behavioral Problems and Competencies of Clinic-referred and Nonreferred Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Achenbach	Research Psychologist	LDP	NIMH
Other:	C. Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)
25 Child Guidance Clinics and other mental health agencies

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.20	.20	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to compare the behavioral problems and competencies reported for clinic-referred and nonreferred (normal) children, a survey of 1300 randomly selected parents in the Washington, D. C. area was conducted. Home interviewers administered the Child Behavior Checklist to parents of 50 boys and 50 girls at each age 4 through 16. The results are being compared with parental reports of problems and competencies for clinic-referred children matched to the nonreferred children for age, sex, socioeconomic status, and race. The data are expected to provide a unique epidemiological baseline for research on child psychopathology and the effectiveness of various treatments in bringing a child's behavior into the normal range for his age.

PHS-6040
(Rev. 10-76)

Project Description:

Although there have been a few normative studies of behavior problems in nonclinical groups of children and other studies of problems in clinical groups, there have been no systematic comparisons of problems and competencies reported for broad samples of clinic-referred and nonreferred children using the same instrument during the same period of time. Comparisons of this sort are badly needed in order to determine what kind of problems and competencies typically differentiate "normal" children from those who are recognized as being disturbed enough to warrant professional attention. Because types of problems and competencies are likely to differ with the age, sex, race, and socioeconomic status of the child, the study is designed to compare referred and nonreferred children matched for these variables at each chronological age from 4 through 16.

The data on the referred children have been collected with the Child Behavior Checklist administered in some 25 child mental health settings in the course of developing the Child Behavior Profile described in the Annual Report on Project No. Z01 MH 00244-02 LDP. The data on the nonreferred children were collected by selecting addresses at random within census tracts stratified so as to obtain a distribution similar to the distribution of clinic cases with respect to socioeconomic status and race. Letters describing the survey were mailed to the selected addresses and recipients were asked to return a postcard if they had no children aged 4 through 16 living with them. An interviewer then went to each address from which no postcard was returned. If a parent consented to be interviewed and if more than one child between the ages of 4 and 16 resided there, a random number table was used to select the child to be described on the Child Behavior Checklist, excluding any who had received mental health services within the previous year.

The scores obtained by the nonreferred boys on all behavior problem and social competence scales of the Child Behavior Profile have been found to differ at highly significant levels from those of clinic-referred boys within each age and socioeconomic group. Although lower socioeconomic parents reported more problems and fewer competencies on some scales than did upper socioeconomic parents, these differences were far smaller than the referred versus nonreferred differences across socioeconomic strata. Similar comparisons will be made on the Child Behavior Profile scales for girls when the Profiles for girls have been completed. Comparisons for both sexes on specific problems show highly significant differences between referred and nonreferred children for most problems, but the problems that best discriminate the groups vary greatly with the age and sex of the children. It is expected that these data will provide a unique epidemiological baseline for further research on child psychopathology and on the effectiveness of various treatment approaches in bringing a child's behavior into the normal range for his age.

Publications:

The Child Behavior Profile: I. Boys Aged 6 through 11. J. Consult Clin. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00264-01 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Child Rearing Correlates of Young Children's Altruism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Zahn-Waxler — Research Psychologist — LDP NIMH

M. Radke-Yarrow — Chief, LDP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Mothers' behaviors were examined in relation to children's early manifestation of altruistic behavior. Sixteen mother-child pairs were studied, over a nine-month period. Children were 1½ to 2½ years of age. Mother-child interactions were observed in the home. Mothers trained in observation techniques reported their children's responses to others' distress, and their own responses in these circumstances. Some distress events were naturally occurring, others were simulated. Maternal behaviors significantly positively associated with children's altruism were sensitive nurturant attention to the child's own distresses, modeling of comfort to other's distress, interpretations of the other's distress state, and explanations and guilt induction when the child was the cause of distress. Mothers treated distresses caused by boy and girls differently; more information about the consequences of hurting others was directed toward boys, while modeling of nurturance to others in distress was more frequently done in the presence of girls. In identifying some of the dimensions of the early child-rearing associated with children's sympathetic behavior, the study provides further information about the origins of altruism.

PHS-6340

Project Description:

Children's sensitivities to affect are assumed to be fashioned, in part, from experiences with the emotions of others. But the effects of different kinds of encounters with others' emotions on children's behavior are largely unknown. The environmental forces that presumably shape children's sensitivities, particularly their compassionate, altruistic reactions to others' distress emotions, are considered in this investigation. The behaviors that caretakers model, teach, and reward or punish in emotional contexts may influence children's altruistic learning: For example, when the mother comforts her own or another child, she provides a good model of how to handle distressful circumstances; or, when she disciplines her child for causing hurt, she conveys various messages about the painful consequences of distress. These and other child-rearing techniques are examined in relation to individual differences in children's expressions of concern for others.

The subjects are 16 pairs of mothers and their children who range in age from 1½ to 2½ years. They are from white, predominantly middle to upper-middle class families. They are part of a developmental study of children's reactions to others' emotions (project report no. Z01 MH 00242-03 LDP).

Data on mothers' child-rearing behaviors were obtained from the following sources: (1) Over a period of nine months investigators made home visits at 3-week intervals and observed mother-child interaction. (2) Mothers were trained to observe and record their child's and their own reactions to naturally occurring distress events such as sorrow, pain, discomfort. Events included distresses caused and not caused by the child. Mother-child interactions were rated on mother's sensitive attention to the child's own distress circumstances, her permissiveness, and her physical affection. Mother's responses were coded on: (a) comforting of victims of distress, (b) interpretations to child of the content of others' distress experiences, and (c) disciplinary techniques used when the child caused distress. Children's responses to distress were coded as altruistic (e.g., helping, hugging, sharing objects, sympathizing, protecting), or aggressive. Satisfactory reliabilitites were obtained on most mother and child scores. All scores were composites based on samplings of mother and child behaviors over a 4 to 5 month interval. Correlational analyses were done using (a) mother and child scores based on the same time period and (b) maternal scores that temporally preceded child assessments.

Maternal behaviors significantly positively associated with children's empathy and altruism were mothers' sensitive, nurturant attention to the child's needs, mothers' provision (modeling) of comfort to others in distress, mothers' interpretations to the child of the other's distress, and the use of explanations and guild indiction when the child was the cause of distress. Maternal behaviors unrelated to child altruism were permissiveness, physical affection expressed in nondistress circumstances, verbal prohibition and physical punishment for causing distress. Mother's maternal rearing

behaviors were significantly correlated across time periods, indicating that mothers have some stable practices for this aspect of rearing. Implicit in the relatively frequent use of explanations to children about the consequences of hurting is the assumption by mothers that children, before age two, understand causal relations; that is, that they are able to distinguish distresses for which they have been responsible from those not caused by them. Mothers use explanations about others' distress experiences mainly when the child has caused the distress.

Mothers gave more interpretations and were more reactive to boys who caused distress to someone than they were to girls, even though boys and girls did not differ in the frequency of caused-distress. Mothers modeled comfort and nurturance to others in distress more often in the presence of girls than boys, suggestive of early training in nurturing behavior.

Empathic and altruistic responses appearing in the first years are probably largely a function of maturation. Very soon, however, there are variations in the degree to which children show concern for another's needs. Some of these individual differences can be accounted for by different learning experiences. Since caring about others is a component of emotional well-being, it is important to identify those experiences that foster empathy and altruism in some children and inhibit it in others. The present study identifies some of the factors operating early in the child-rearing process, and, in so doing, it contributes to understanding the origins of empathy and altruism.

A manuscript is being prepared.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00265-01 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chronic Malnutrition and Child Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief, LDP NIMH	LDP NIMH
	David E. Barrett	Staff Fellow	LDP NIMH
OTHERS:	Prabha Ramalingaswami	Asst. Professor	Nehru University New Delhi, India
	Albert Hollenbeck	Staff Fellow	LDP NIMH
	Elizabeth Susman	Visiting Scientist	LDP NIMH
	Carolyn Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Psychology

CITY & STATE

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

INTRAMURAL:	EXTRAMURAL:	OTHER:
.50	.25	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is an investigation of the effects of chronic malnutrition on social, cognitive, and affective behavioral development of children. Six and seven year old children, identified as chronically malnourished, will be provided daily protein and calorie supplements. Children from experimental groups receive food supplements for two consecutive 14 month periods; children from control groups receive supplements beginning in the second intervention period. The criterion behaviors include attentional capacity, memory, and problem-solving; emotional responsivity and impulse-control; and patterns of social interaction. Periodic assessments of health and nutritional status are made. Differential effects of food supplement on children not malnourished will also be determined.

Project Description:

Chronic malnutrition is a condition of existence for many millions of children. Its most characteristic contexts are the environments of poverty and disease, with the inherent hazards and deprivations that they present. Although it is difficult to disentangle the influences of nutritional status from other factors, a body of reliable findings documents the effects of malnutrition on the structural integrity of the organism. Less well understood are the consequences of malnutrition for the functioning of the organism. Hard evidence comes primarily from animal research. Passivity and apathy and an inability to sustain attention are widely reported, as are reduced exploration and curiosity, fearful avoidance of new stimuli, failure to respond normally to social initiations from other animals, all of which would appear to indicate a functional isolation from environmental stimulation. Malnourished animals show more nonpurposive behavior, more excitability and emotionality, and manifest more unpredictable aggression. In studies of malnourished animal infants and their mothers, disturbed interactions are reported. These behavioral impairments comprise a critical complex of behavioral dimensions. In the context of human development, such impairments would have significant implications for cognitive development and for the social-emotional functioning of the child.

In this research, the focus is on the functional integrity of chronically malnourished children, on the social, cognitive, and emotional development of children. The design provides for assessment of the behavior of malnourished children and of the effects of food supplement on their behavior.

Subjects are six and seven year old children identified as chronically malnourished. (There is also a subsample of non-malnourished children.) The approach is experimental. The design is one of lagged experimental treatments, in which children in all conditions receive the nutritional supplement, but there is a difference in when they receive it. A protein-calorie supplement is provided over a two-year period. There are four experimental groups of malnourished children. Subjects in groups 1 and 3 are measured on the dependent variables at the "baseline" period (time 1). Groups 2 and 4 are not measured at this time. Groups 1 and 2 begin receiving the nutritional supplement immediately after the first assessment period. They are reassessed at time 2 and again at time 3. Groups 3 and 4 are measured at time 2, prior to nutritional intervention. For these groups, nutritional supplements begin following the time 2 measurement period. Both groups are remeasured at time 3.

In addition to the main groups deaignated, a subsample of non-malnourished children from the same population will be studied (groups 1_n and 3_n). Also in order to control for the possible effects of social stimulation alone, a group 3_s will be studied; these children will have social stimulation between measurement period time 1 and time 2.

For the children in the study, the added food will be given in a supervised setting. Some food supplement is given to other family members but is not supervised.

Assessments of the children will include (1) background data on health, nutritional status, and family characteristics, and also assessment of current status in these respects, (2) monitoring of food intake and assessment of parent-child interactions, (3) attentional and cognitive measures, and (4) affective and social measures, on the dimensions indicated in the statement of the problem. Measurement will take place both in individual sessions of testing and observation and in small-group sessions consisting of children who will come together for successive, experimentally-designed observation periods.

The study will be carried out in an area of extreme rural poverty. Several sites are currently under consideration.

Current work is proceeding (a) on the necessary collaborations outside the Laboratory to make this field study possible, and (b) on the development of the measures and on pretesting of the measures.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00266-01 LDP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Newborn Interaction with Mothers and Nonmothers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz	Research Psychologist	LDP, NIMH
OTHER:	Robert L. Manniello	Assoc. Investigator	NICHHD
	Elizabeth F. Boyd	Assoc. Investigator	Univ. of Maryland Baltimore County

COOPERATING UNITS (if any)

Alexandria Hospital, Alexandria, Virginia

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aims of this investigation are fourfold: (1) To investigate the effects of different types and durations of contact (skin-to-skin, cradling) between mother and her newborn in the first post-partum hour on the mother's attachment to her infant, their interaction, and the infant's behavioral development, at 3 and 28 days of age; (2) To investigate the effects of two durations of contact between father and his C-Section newborn in the first 1½ hours post partum on the father's attachment to his infant, their interaction, mother's interaction with infant, and infant's behavioral development, at 4 and 28 days of age; (3) To determine if a differential attachment to a newborn infant at 3 days can be fostered in nonmother (nursing student) by a program of intense interaction with that newborn during its post-partum hospital stay; (4) To determine if,at 3 days, the quality and quantity of interaction of a newborn with its mother, and its behavioral development (as indexed by the Brazelton Neonatal Assessment Scale), can be fostered by a concurrent program of intense interaction between that newborn and a nonmother interactor. Twenty second-year nursing students, 60 full-term newborn infants and their mothers, and 30 C-section-delivered newborns and their fathers and mothers, will serve as subjects in this study.

PHS-2040

Project Description:

The aims of this project are:

1. To investigate the effects of different types and durations of contact (skin-to-skin, cradling) between the mother and her newborn in the first post-partum hour on the mother's attachment to her infant, their interaction, and the infant's behavioral development, at 3 and 28 days of age;

2. To investigate the effects of two durations of contact between the father and his Caesarian-Section-delivered newborn in the first 1½ hours post partum on the father's attachment to his infant, their interaction, the mother's interaction with the infant, and the infant's behavioral development, at 4 and 28 days of age.

3. To determine if a differential attachment to a newborn infant can be fostered in nonmothers (nursing students) by a program of intense interaction, beginning at birth, with that newborn during its post-partum hospital stay; and

4. To determine if, at 3 days, the quality and quantity of interaction of a newborn with its mother and its behavioral development can be fostered by a concurrent program of intense interaction between that newborn and a nonmother (nursing student) interactor.

The immediate post-partum mother-infant and father-infant contact manipulations are relevant to issues in the development of parental attachment to infants. Recent work has drawn attention to the fact that skin post-partum contact between neonate and mother in the first hour (de Château, 1976), and contact opportunity during the first three days (Klaus, Jerauld, Kreger, McAlpine, Steffa, & Kennell, 1972), could result in maternal behaviors denoting attachment to the infant, both in the neonatal and subsequent periods. These remarkable findings have directed attention to the importance of mother-infant interaction in the first post-partum hours.

At the same time, caregiver-infant interaction has been increasingly the focus of research on the social development of the child. The young infant's limited repertory of communicative behaviors (e.g., babbling, crying, smiling) form the basis of his/her communication with the caregiving environment. These infant behaviors are thought to have a unique potential for influencing caregiver behaviors and thus may constitute a key to understanding the development of maternal or paternal attachment to the infant (Gewirtz & Boyd, 1977) and to determining the conditions under which a mother or nonmother caregiver might acquire an attachment to an infant. At the same time, the way the infant acquires patterns of communication with its mother/caregiver can facilitate an understanding of the origins of the early attachment of the infant to its mother as well as of the ways the infant learns to control the caregiving environment (Gewirtz, 1976). At present, few hard data are available on either the influence processes or the outcomes involved.

The manipulations proposed in this investigation have the primary purpose of replicating and extending, in a controlled setting, studies of the possible

effects of immediate post-partum mother-infant contact on the development of maternal behaviors denoting attachment to the infant, and of identifying some features of parent- and nonmother-infant interchange that can account for the development of parent and nonmother attachment to the infant.

Mothers of full-term infants, vaginally delivered without complication under epidural anesthesis, and their normal infants will serve as subjects under Aims 1, 3, and 4. Mothers will receive instructions to guide their interactions with their newborns in the recovery room (Aim 1) or will be approached then to permit interaction with, and observation of, their infants in the nursing-student study (Aims 3 and 4). Infants delivered without complications via routine C-Section procedures, and their fathers and mothers, will be the subjects under Aim 2. Fathers will be given instructions to guide their interaction with their newborns right after delivery in an area set aside in the special-care nursery. Under all aims, infants will be assessed as normal full-term newborns with Apgar scores >8 at five minutes of age. Infant temperatures will be taken initially and at 15-minute intervals during periods neonates are with mothers during the first post-partum hour. In no case will a mother or infant spend less time in the recovery area than is usual at the Alexandria Hospital (60 minutes for mothers, 15 minutes for neonates). Assignment of parent-infant pairs or infants to treatment conditions will be done at random.

For Aims 3 and 4, some 20 second-year nursing students, from two schools of nursing, apprenticing at the Alexandria Hospital will serve as subjects. These nursing students will have the impression that the focus of the study in which they are to participate will be on whether or not intensive interaction of caregivers with newborns ("primary" infants) could foster the development of newborn social behavior (Aim 4). Under Aim 3, each nursing student subject will be assigned a primary (experimental) and a secondary (control) infant for the 2-3 days of their service. The intensive interaction of each nursing-student with her primary infant, that constitutes the treatment condition under Aim 3, is governed by a script that calls for repeated tactile and auditory stimulations, and repeated assessments of consolability, cuddliness, and visual fixation of the infant (according to the Brazelton Neonatal Behavioral Assessment Scale). In addition, the student nurse will keep a running diary of the primary infant's states, responses to stimuli, and behavioral assessments.

Under Aim 1, mother-infant contact in the recovery room during the first post-partum hour will occur under one of three conditions: 1) 60-minute ventral-ventral skin-to-skin contact, where the naked newborn is placed on mother's abdomen, is supported by her in an encompassing manner, and covered with a blanket (to minimize heat loss); 2) 15-minute cradling, where the newborn, wrapped in a blanket, is nested in the crook of its mother's elbow; and 3) 60-minute cradling, were, as in 2, the wrapped neonate is nested in the crook of its mother's elbow.

Observations of the newborn with nursing student, mother, or father will be

made, using the following behavior categories: For mother, father, or nursing-student--reposition, smile, enface looking, body looking, talk, vocal non-language sound, making visual spectacle, finger contact, palm contact, rock, hold, kiss, grimace, feed, and imitate; For infant--grimace, smile, enface looking, eyes open, nonvocal sound, vocal sound, mouthing, fuss-cry, and motor movement. In addition, responses of mother, father, nursing student, or child contingent on responses of the other will be scored.

Under Aim 1, an observation of the initial interchange between mother and neonate will be made in the recovery area. In addition, two 15-minute observations of mother and infant, one during a feed and one between feeds, will be made at 48 hours and at 28 days after birth. In addition, an assessment using the Brazelton Neonatal Behavioral Assessment Scale will be made at those two time points.

Under Aim 2, an observation of the initial interchange between father and C-section-delivered neonate will be made right after delivery in the special-care nursery. In addition, one feed and one nonfeed 15-minute observation of mother and infant will be made at 72 hours and at 28 days after birth, and a 15-minute nonfeed observation of father and infant will be made at 96 hours and at 28 days after birth. Also, the infant's behaviors (Brazelton Scale) will be assessed at 48 hours and 28 days.

Under Aims 3 and 4, two daily 15-minute observations of the interchange betw nursing student and primary (experimental) infant, between nursing student and secondary (control) infant, and between those infants and their mothers at 48 hours will be made. In addition, under Aim 4, an assessment of both experimental and control infants at 48 hours using the Brazelton Scale will be made.

The investigations under the aims of the program outline are in their beginning phases.

Publications:

None

[illegible] RESEARCH NUMBER (Do not [illegible])	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00267-01 LDP

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Impact of Television on Families with Young Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Hollenbeck	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)

University of Washington, Seattle, Washington

LABORATORY

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.40	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) [illegible] ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As part of a larger study on the influence of television on infants' visual and vocal behavior, family patterns of television viewing were assessed using multiple measures and a measure of mother-infant relations was validated. Study A explored different methods of assessing television viewing patterns and examined the relationship between parental viewing and infant exposure to television. Results suggest that estimates of television viewing time exceed the quantity of television actually viewed. In addition, infant exposure to television in the home was related to mother's age but not SES. Study B tested the concurrent validity of the Home Stimulation Inventory (Home), a measure of the quality and quantity of social, emotional and cognitive support in the home. Results indicated the validity of the Home as a measure of early infant environments.

Project Description:

As part of a larger study on the influence of television on infant's visual and vocal behavior (Hollenbeck, 1976) data were collected on television viewing patterns of families and on mother-infant relations using the Home Stimulation Inventory.

Study A: Television viewing patterns of families with young infants.

The impact of television on the American way of life has been well documented; however, little research exists on the effects of television on children under three years of age. It has been suggested that family members influence one another's viewing habits. Specifically, viewing patterns of children have been related to parental viewing patterns. In addition, evidence indicates that parents put few restrictions on children's viewing. These findings suggest that one index of the viewing patterns of children under three years of age is the viewing patterns of their parents. However, exact estimates of viewing patterns, even for adults, are difficult to establish. Different methods of assessment have proved unreliable.

The purpose of this study was two-fold: (1) to compare different methods of assessing television viewing patterns,and (2) to examine the relationship between parental viewing and infant exposure to television in the home. Sixty parents kept diary records of television programs watched for one week, recalled programs watched in a previous week, and estimated the number of hours of television they watched per day. Results indicate that estimates were significantly longer than either diary records or recall measures, which did not differ. In addition, infant exposure to television in the home (2 hours per day) was correlated with mother's age, but not socio-economic-status of the family. Results are discussed in terms of the relative merits of different methods for assessing television viewing patterns and specific relationships between parental viewing habits and subsequent infant exposure to television.

Study B: Early infant home environments.

The general dissatisfaction with broad indices of socio-economic-status has led to the development of more analytic process-oriented measures of the environment. One of the more promising scales designed to assess subtle processes in children's environments is the Home Stimulation Inventory (HOME) developed by Caldwell, Heider and Kaplan. This inventory was "designed to sample certain aspects of the quantity and quality of social, emotional, and cognitive support available to a young child (birth to three years) within the home (Caldwell, Heider and Kaplan). Numerous studies by the originators and others demonstrate that HOME environment scores are related to cognitive development in early childhood. However, few studies have reported measures of validity for the HOME, especially for younger infants. Moderate correlations (.25 - .55) have been reported between HOME scores and several socio-economic indicators. To date, extensive standardization and validity data for the inventory do not exist for young infants.

Scores from the Home Stimulation Inventory from a sample of 70 mother-infant dyads were used to cross validate and test the concurrent validity of the HOME. The HOME was significantly correlated with several indicators of socio-economic-status. In addition, scores on the HOME varied in predictable directions, given age and class differences, between two standardization samples and the new sample. This independent replication validates original correlations between the HOME and social indicators found in a standardization study. Evidence between groups suggests that the HOME is a viable instrument able to discriminate between diverse populations in predictable ways.

Data on these projects were collected prior to appointment in the Laboratory. Time in the Laboratory was devoted to analytic work on these studies. A manuscript has been prepared, "Early infant home environments: Validation of the Home Stimulation Inventory," and submitted to Developmental Psychology.

Publications:

Hollenbeck, A. R. Television viewing patterns of families with young infants. J. Soc. Psychol., in press 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00268-01 LDP

PERIOD COVERED
July 1,1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Problems of Reliability in Observational Research--A Methodological Analysis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. R. Hollenbeck Staff Fellow LDP NIMH

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University of Washington, Seattle, WA

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.60	.10

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Major advances have occurred in the last ten years in techniques for observing behavior under natural conditions. However, limited attention has been given to traditional psychometric issues of validity and reliability. The purpose of this analysis was to provide a guide to literature on reliability in observational research. Current problems in definition and theory, practical applications, and sequential analysis were explored.

Project Description:

In recent years the use of observational methods in nonfield settings appears to be increasing in the developmental, social, and personality areas of behavioral sciences. As the use of observational techniques in all settings becomes more focused and precise, the need to evaluate observational methodology becomes more apparent. Do these techniques actually accomplish the scientific tasks we set for them? At the present time, few observational scoring systems would meet measurement standards such as reliability that we use in evaluating other psychological measures. This project attempts further to illuminate problems of assessing reliability in observational research and to suggest approaches to solving some of these problems.

The purpose of this project was threefold: First, to provide a guide to the literature on reliability in observational research. Second, to examine commonly used coefficients of agreement, their applicability and their relative merit. Third, to examine attempts to extend reliability theory to sequential data analysis.

Definition is a problem. Meanings given to terms such as "agreement," "accuracy," "precision," "stability," and even "reliability" vary in practical usage from author to author. The confusion in terminology, however, is only symptomatic of the failure to integrate traditional psychometric theories of reliability with a theory of observation. Most users of observational systems recognize the need for reliability, yet most only assess and report observer agreement--usually a poor estimate of reliability.

A second problem is associated with the observing unit or the person making the observations together with the systematic coding scheme used to record observations. Issues in this area include the types of data generated (frequency, duration, time sampling, or sequential), the level of units in the coding system (e.g., molar or molecular), the complexity of the coding system, concepts of observer and observer bias, circumstances of assessment, and observer drift and decay. A third problem area is the practical one of choosing a statistical index of agreement. There are at least seven variations, all of which suffer from one problem or another limiting their usefulness.

Reliability should be defined in terms of accuracy and stability, and not just the accuracy or stability of a measure. Neither alone is sufficient. Reliability can only be established through an analysis of measurement error or its nonparametric equivalent. Observer agreement means agreement and not necessarily accuracy, stability, or reliability. However, observer agreement may serve as an index of reliability when a prior relationship between agreement and reliability has been demonstrated for a particular scoring system. Thus, agreement scores may be the final statistic once a scoring system has demonstrated reliability. The degree to which reliable scoring systems may be applied using different observers, different situations, and different observable populations needs to be explored experimentally.

A scoring system should be examined for reliability by providing the method used to examine the system for reliability and by reporting actual reliability estimates when a scoring system is first applied and described in the literature. Reports of subsequent use of the scoring system should reference this evidence and demonstrate how indices of agreement, for example, justify use of the scoring system in the new situation. The index of agreement should be comparable across studies. Robinson's A and Cohen's kappa statistics both correct for chance levels of agreement and are suitable at different levels of measurement. Until the reliability of observational measures is spelled out in the scientific literature, the validity of observational data will remain suspect. This is a final report.

Publications:

Hollenbeck, A. R. Problems of reliability in observational research. In G. P. Sackett (Ed.), Observing Behavior, Volume 2: Data Collection and Analysis Methods, Chapter 5. University Park Press: Baltimore, in press 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00478-21 LN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Research Physiologist	LN NIMH
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	R. Nakamura	NIMH Extramural Fellow	LN NIMH
	L. Ungerleider	NEI Extramural Fellow	LN NIMH
	C. Gross	Professor	Princeton University
	B. Turner	Professor	Howard University
	C. Kennedy	Neurologist	LCM NIMH
	M. Buchsbaum	Psychiatrist	AP NIMH

COOPERATING UNITS (if any)
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Howard University — Adult Psychiatry Branch, NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.4	2.8	3.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate cortex is the source of two major, corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnecting the striate, prestriate, posterior parietal, and dorsal prefrontal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, inferior temporal, and ventral limbic areas, and is critical instead for object vision. Our research concerns both pathways, but the accompanying report focuses on the second, or ventral one. Our studies suggest that each of the extrastriate links in this ventral pathway adds a different capability to object vision, making possible, in turn, highly efficient object perception, object memory, and object-affect associations. The sequential dependency of these behavioral functions is paralleled by the sequential dependency of the successive neural structures in the chain.

1. Project Description:

The objective of the eight sub-projects described here is to define the role in object vision of neural structures outside the primary visual projection system, using behavioral and anatomical analyses. (1) Monkeys are prepared with various lesions of the medial (peripheral) striate cortex and the brains are then studied with the Fink-Heimer silver impregnation method for determining the cortical projections from this previously unexamined area. The results will make it possible to complete the map of the striate-prestriate projection system. (2) Monkeys are prepared with as complete ablations as possible of the striate-receptive zone defined in (1) and trained on visual pattern discriminations. If learning occurs, the animals are given a second lesion in which all the tissue in one hemisphere caudal to the original lesion is ablated, and the brains are then studied by the silver impregnation method for degeneration in the IT area. The results will provide a rigorous test of the hypothesis that the prestriate cortex serves as the link between the striate cortex and the inferior temporal cortex. (3) Monkeys with combined inferior temporal lesions and optic chiasm sections were trained on a series of visual pattern discriminations, each presented first to one eye and then to the other. Unlike control monkeys (with either chiasm section alone or temporal lesions alone), the experimental monkeys failed to show interocular transfer. The results suggest that stimulus equivalence between the visual half-fields depends on the convergence of their projections onto single inferior temporal neurons through a striate-prestriate-temporal pathway that also involves the forebrain commissures. Follow-up work is exploring whether inferior temporal neurons account for stimulus equivalence across any retinal translation, and also whether, as suspected, it is the neurons in the posterior part of inferior temporal cortex that are mainly responsible for this stimulus equivalence mechanism. (4) Monkeys with various cerebral lesions were compared on tests designed to measure one-trial visual memory for objects. Only removals within the anterior part of inferior temporal cortex have impaired this object recognition ability, suggesting that this is the locus in which the visual trace left by previous viewing is preferentially established. (5) Monkeys are prepared with various lesions of the inferior temporal cortex and the brains are then impregnated with the Fink-Heimer silver stain to study the pathways leading out of the inferior temporal visual area. Preliminary results indicate that there are strong projections from the anterior inferior temporal cortex to several limbic regions (amygdala, hippocampal gyrus, orbital frontal gyrus) and to the striatum (posteroventral putamen and tail of caudate). (6) Monkeys with lesions in the projection areas defined in (5) are compared on tests designed to measure one-trial visual associative memory. Preliminary results suggest that, among the several limbic lesions, amygdalectomy produces the most severe impairment. Further work will investigate whether "disconnection" of the amygdala from its inferior temporal visual input will reproduce the associative memory deficit seen after amygdalectomy. (7) A new way to investigate the entire pathway with which this project is concerned became

available recently with the development, by Sokoloff, Kennedy and their colleagues, of the [^{14}C] deoxyglucose method. In collaboration with Kennedy, we have used this method to compare the uptake of labelled deoxyglucose in a blind and a seeing hemisphere of a split-brain monkey. Differerential uptake in the two sides permits the tracing of the entire visual pathway, from striate cortex to limbic system, as described in this project. (8) Evidence from the other sub-projects has suggested that a telencephalic pathway from striate to prestriate to inferior temporal cortex and to the limbic lobe is necessary for visual discrimination learning. To test whether this pathway is also sufficient for this function, monkeys were prepared with forebrain commissurotomies combined with unilateral removals of all cortex except for the postulated visual-discrimination-learning pathway. Preliminary indications are that the lesion produces a longlasting, functional blindness. We are currently investigating the mechanisms responsible for this unexpected result.

Scientific Significance to Mental Health Research. Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events which intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impariment in visually-mediated behavior has opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision should aid greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

2. Publications:

Blake, L., Jarvis, C. D. and Mishkin, M.: Pattern discrimination thresholds after partial inferior temporal or lateral striate lesions in monkeys. Brain Research 120: 209-220, 1977.

Delacour, J.: Role of temporal lobe structures in visual short-term memory, using a new test. Neuropsychologia 15: 681-684, 1977.

Elliott, R. C., Norris, E., Ettlinger, G. and Mishkin, M.: Some factors influencing nonmatching to sample in the monkey. Bulletin of the Psychonomic Society 9: 395-396, 1977.

Gross, C. G. and Mishkin, M.: The neural basis of stimulus equivalence across retinal translation. In Harnad et. al. (Eds.) Lateralization in the Nervous System. Academic Press, New York, 1977, pp. 109-122.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00480-13 LN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of brain and behavior in primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Research Physiologist	LN NIMH
OTHER:	H. E. Rosvold	Chief, Laboratory of Neuropsychology	LN NIMH
	R. M. Brown	Sr. Staff Fellow	LN NIMH
	G. E. Alexander	Commissioned Officer	LBB NICHD
	T. J. Brozoski	Guest Worker	LN NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.2	3.2	4.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major goal of this laboratory is the elucidation of neural mechanisms underlying the development of normal behavior and equally to understand the mechanisms responsible for recovery of function and plasticity following central nervous system injuries, particularly those originating in infancy. The approach is broadly multidisciplinary, involving behavioral, anatomical, electrophysiological, endocrinological, pharmacological and biochemical techniques. The experimental subject is the rhesus monkey, which because of its protracted span of postnatal development and its remarkable capacity for restitution of function following cortical lesions of early onset is unsurpassed as a model of normal and disordered human development. The focus of these studies is on the prefrontal granular cortex, a slowly maturing neocortical region of the primate brain particularly appropriate for studies on the sequence, duration, and correlation of structural and functional events in ontogeny. The emphasis of this research is reflected in the titles of the four subprojects; (1) Cryogenic Depression of Cortex and Subcortical Unit Activity at Different Stages of Postnatal Develop-

ment. (2) Sexual Dimorphism in Cortical Structure and Function. (3) Behavioral and Anatomical Consequences of Prenatally Induced Brain Damage. (4) Neurochemical Correlates of Normal and Disordered Development in Primates.

1. Project Description:

A major goal of this laboratory continues to be the elucidation of neural mechanisms underlying the development of normal behavior and equally to understand the mechanisms responsible for recovery of function or plasticity following central nervous system injuries, particularly those originating in infancy. The approach is broadly multi-disciplinary, involving behavioral, anatomical (to be described in a separate project), electrophysiological, endocrinological, pharmacological and biochemical techniques. The experimental subject is the rhesus monkey, which because of its protracted span of postnatal development and its remarkable capacity for restitution of function following cortical lesions of early onset, is an ideal subject for studies aimed at understanding neurobehavioral specificity and plasticity. Moreover the suitability of the primate as a model of normal and disordered human development is unsurpassed. The focus of these studies is on the prefrontal granular cortex. This slowly maturing neocortical region of the primate brain is particularly appropriate for studies aimed at resolving the sequence, duration, and correlation of structural and functional events in ontogeny.

Cryogenic Depression of Cortex and Subcortical Unit Activity at Different Stages of Development. Our previous work in this laboratory has provided evidence that specific regions of the primate cortex are functionally immature at birth and develop their functions according to widely different timetables. However, the evidence indicates that developing monkeys differ from adults not only with respect to cortical immaturity but also with respect to cortical regulation of subcortical structures and functions. With Dr. Gary Alexander, we have employed cryogenic cooling and electrophysiological recording techniques to further describe the changes in cortical-subcortical organization that take place in ontogeny. Previous studies by Dr. Alexander have shown that the frontal cortex in adult monkeys can be temporarily inactivated by cooling the cortex through stainless steel cryoplates implanted over the dura mater. In the adult monkey, localized cooling of the prefrontal cortex results in behavioral impairments which typically follow ablation of the same cortex but the effects are reversible, i.e., performance returns to criterion level when the cortex is returned to its normal temperature. Furthermore, cooling has been shown to inhibit the firing of neurons in the dorsomedial nucleus of the thalmus in association with behavioral performance. Studies identical to those that have been carried out in mature monkeys are now being conducted in normal monkeys at various stages of postnatal development. Our studies show that cryogenic depression of the dorsolateral prefrontal cortex at a temperature sufficient

to induce 21-25% decrements in delayed response performance in 34-36 month old monkeys, produced deficits of 7-8% in 19-31 month olds and no detectable loss in the youngest monkeys, 9-16 months of age. The results obtained with the reversible lesion procedure in normal monkeys are remarkably similar to those obtained in monkeys with permanent brain damage and thus provide evidence that cortical cooling is a powerful approach to the study of regional maturation in the primate brain. Moreover, the consistent results observed with both the ablation and cooling procedure provides strong evidence that the dorsolateral cortex does not become fully functionally mature until the end of the second year of life, i.e., close to puberty. Electrophysiological data have been collected and are now being analyzed to determine whether striatal or thalamic unit activity in infants is modified with the same degree of specificity as it is in the adult. The results of the electrophysiological studies in behaving monkeys at different stages of development should reveal the extent to which subcortical mechanisms can function more autonomously in the young than the old, a principle of central nervous system organization in normal development suggested by previous research, and one which could help to explain the remarkable sparing of function observed after early cortical injuries.

Sexual Dimorphism in Cortical Structure and Function. Previous work in this laboratory has provided evidence that the orbital prefrontal cortex develops function considerably earlier in males than in females. Currently we are studying the behavioral and biochemical consequences of postnatal injections of testosterone in developing female monkeys to determine whether cortical differentiation can be influenced by gonadal hormones at early stages of development. Performance on a cognitive task known to require the integrity of the orbital prefrontal cortex in the adult was studied in normal 75-day old male and female rhesus monkeys and in 75-day old females administered a total of 38.5 mg/kg of testosterone proprionate in multiple injections from birth through 46 days of postnatal development. At this age normal males perform at significantly higher levels than do normal females. Postnatal injections of testosterone proprionate produced slight but permanent alterations in the morphology of the external genitalia and, on the cognitive task, enhanced the performance of the adrogenized females to the level of normal males. Additional groups of males and androgenized females were subjected to bilateral orbital prefrontal lesions at 50 days of age and tested at 75 days of age. These groups exhibited deficits on the cognitive task whereas nontreated females that were given identical lesions and tested at the same ages did not differ from unoperated females. These results, together with previous findings in older monkeys (Goldman, et al., Science, 186: 540, 1974), provide evidence that (1) the orbital prefrontal cortex develops at different rates in males and females; (2) androgen accelerates the development of orbital prefrontal function, suggesting that gonadal hormones may play an inductive role in the differentiation of cortical mechanisms similar to that played in the differentiation of

hypothalamic mechanisms; and (3) the critical period for the influence of androgen on function of the central nervous system in primates extends into postnatal life.

The possibility that there are neural differences between males and females at the cortical level under the control of gonadal hormones at critical periods in development is of extreme importance for understanding the normal differences in cognitive and social development of the two sexes as well as for understanding disturbances of psychosexual orientation such as adrenogenital syndrome and progestin-induced pseudohermaphroditism that may occur as a consequence of exposure to an inappropriate hormonal milieu during gestation. Future plans for work in this area include behavioral and biochemical studies of prenatally-androgenized females and prenatally feminized males (chemically gonadectomized with maternal injections of cyproterene acetate, an androgen antagonist). Prenatal studies are warranted because the peak sensitivity of cortical tissue to gonadal hormones in the primate may be expected to coincide with the critical period for cortico-genesis in the second trimester of gestation.

Behavioral and Anatomical Consequences of Prenatally-Induced Brain Damage. The presumptive dorsolateral prefrontal cortex was resected bilaterally in a fetal rhesus monkey at E106 (106th embryonic day) with subsequent replacement in utero and caesarean delivery near term (165 days). Cognitive functions dependent upon the integrity of this neocortical region were assessed during postnatal development. The monkey was sacrificed at 2½ years of age and its brain fixed by vascular perfusion and processed for subsequent cytoarchitectonic and hodological analysis. The prenatally operated monkey grew to 2½ years of age without exhibiting any gross sensorimotor deficits or evidence of impairment on the classical spatial delayed response problems which depend critically upon the integrity of the dorsolateral prefrontal cortex in mature monkeys. Its performance on these tests was within the range of unoperated monkeys rather than severely impaired as in the case of monkeys with the same cortical areas removed as juveniles or adults. The lesion performed at E106, when brain fissuration has barely begun, were bilaterally symmetrical and similar in size and topography to those performed after birth when the brain has attained the adult fissural pattern. However, the surface of the telencephalon in the monkey operated upon as a fetus exhibited marked changes in gross morphology; anomalous sulci appeared bilaterally on the lateroventral surface of the frontal lobe and some unusual sulci were also observed in locations as remote as the occipital lobe. The cytoarchitectonic composition of the cortex lining these sulci displayed the basic six-layered pattern of primate neocortex. Perhaps the most significant finding with regard to a possible explanation of functional plasticity is that the parvocellular subdivision of the mediodorsal thalamic nucleus, which normally projects to the dorsolateral prefrontal cortex, shows little evidence of neuron loss, indicating perhaps that the parvocellular

neurons have found some other cortical target for projection. This sector of the dorsomedial nucleus always degenerates following comparable prefrontal lesions performed after birth. Thus, removal of the dorsolateral prefrontal association cortex in a nonhuman primate a full two months before birth can be followed in postnatal life by extraordinary preservation of behavioral function as well as by significant alteration in the gross morphological characteristics and thalamo-cortical connections of the brain. We are currently engaged in studies to determine the precise neuroanatomical mechanisms underlying this striking example of neurobehavioral plasticity.

Neurochemical Correlates of Normal and Disordered Development in Primates. As mentioned, behavioral testing following ablation of the dorsolateral and orbital prefrontal cortical areas in the developing monkey have now shown that these two cortical subdivisions develop their functions according to different timetables. Also the orbital cortex becomes functionally mature earlier in males than in females. We need to know more about the maturational status of the brain at different stages of development in order to fully understand the ontogeny of behavior and neurobehavioral plasticity in the developing primate. Measures of neurotransmitter levels and rates of synthesis give an indication of synaptic mechanisms in normal development and provide a baseline against which to evaluate events following acute or chronic brain damage.

Three monoamine putative neurotransmitters are of interest in these studies: an indoleamine, 5-hydroxytryptamine (serotonin (5-HT)), and two catecholamines, noradrenaline (NE) and dopamine (DA). Normal neurochemical profiles have been measured in various cortical and subcortical structures of rhesus monkeys at 2 days, 8 months, and 2 years of age or beyond. Levels and biosynthetic activity follow different developmental patterns which are specific to structure and amine. For example, the brain stem shows higher monoamine levels and rates of synthesis at birth than during adulthood. In contrast the general pattern of catecholamine development in other subcortical structures such as caudate, putamen, hypothalamus and hippocampus is a gradually increasing catecholamine level from birth to maturity with rates of synthesis increasing in proportion to concentration. The indoleamine, serotonin, on the other hand exhibits higher levels in some of these structures (caudate and putamen) at birth than at 8-months of age or adulthood. In cortex, only prefrontal areas contain an adult concentration of dopamine. The other amines increase in linear fashion from birth to maturity in prefrontal and other regions of the cerebral mantle. It is likely that the high dopamine levels in newborn prefrontal areas reflect maturational events rather than utilization for synaptic transmission.

Monoamines exhibit a differential distribution in the cerebral cortex of both infant and adult monkeys. A major finding is that monoamines are not evenly distributed in the primate cerebral cortex but instead exhibit a definite gradient of distribution specific to each amine. Dopamine concentration is highest in prefrontal cortex and declines linearly in a

rostral to caudal direction. Although serotonin is rather evenly localized, its deaminated product, 5-HIAA, is distributed in almost mirror image fashion to that of dopamine. Thus, the concentration of 5-HIAA is highest in visual cortex and decreases in a caudal to rostral direction. Both DA and 5-HIAA show high concentrations in temporal cortex. In contrast to both DA and 5-HIAA, NE exhibits a more erratic pattern of distribution. A notably high concentration of NE is found in the somatosensory cortex. Anterior inferior temporal cortex and superior temporal cortex also contained substantial amounts of NE which were higher than that in the visual and posterior inferotemporal cortices, on the one hand, and that in the frontal cortex, on the other. There was thus the suggestion of peak concentrations of NE located in the central cortical zones with NE gradients diminishing along both the anterior and posterior axes. Until now there has been little recognition of the differential concentrations and turnover of monoamines in various areas of primate cortex. These findings as well as the evidence for differential rates of development (Brown and Goldman, '77) encourage the detailed examination of the neurochemical basis of higher behavioral processes and their elaboration. In particular, the role of serotonin in visual function, norepinephrine in somatic sensation, and dopamine in cognition need to be explored.

To investigate the hypothesis that the developing brain reacts differently to damage than a fully mature brain, levels and synthesis rates of monoamines in various cortical structures have been measured eight months following bilateral orbital lesions in infant and adult monkeys. The monoamine levels and synthesis rates have been compared to data from normal animals of the same age group. Two major differences in response to damage have so far been observed between infant-lesioned and adult-lesioned animals: 1. At the site of damage, there is an increase in catecholamine synthesis in infant brains, and a decrease in synthesis in adult brains. 2. In thalamus of the infant-lesioned brain, there is an increase in dopamine content and a 50% decrease in serotonin synthesis rate. A different response occurs in the thalamus of adult-lesioned brain. Here dopamine content does not increase and the serotonin synthesis rate increases 200% Further work is in progress to identify the biochemical alterations that occur following brain damage. The findings from such studies may eventually suggest a pharmacological approach to ameliorating some of the symptoms following brain injury in the young and the old.

This year a beginning has also been made to identify the role of biochemical systems in determining cognitive behavior in monkeys. The fact that the surgical removal of prefrontal cortex in the area of the principal sulcus invariably results in impaired performance on the delayed alternation test made it the test of choice as a measure of cognitive behavior; the depletion of endogenous neurochemicals by specific neurotoxins was used to generate changes in biochemical systems. Preliminary data on 11 animals at various stages of training and testing indicate that 6-OHDA- treated animals,

despite massive NE depletion in prefrontal cortex, do not show the gross debilitation in delayed alternation performance seen in surgically lesioned animals. Rather, they show a subtle but consistent degradation of performance. The effect of DA was more impressive, suggesting that DA is the important factor in mediating the cognitive functions executed by the prefrontal cortex while NE is of secondary importance. Other studies involving manipulation of neurochemicals such as desmethylimipramine which prevents the reuptake of catecholamines into noradrenergic neurons while, on the other hand, agents such as d-amphetamine, which restore catecholamines to normal values, may also provide some insight into the role played by neurochemicals in mediating cognitive functions.

2. Publications:

Brown, R. M. and Goldman, P. S.: Catecholamines in neocortex of rhesus monkeys: regional distribution and ontogenetic development. Brain Research, 124: 576-580, 1977.

Goldman, P. S.: Maturation of the mammalian nervous system and the ontogeny of behavior. In Rosenblatt, J. S., Hinde, R. A., Shaw, E. and Beer, C. (Eds.): Advances in the Study of Behavior. Academic Press, New York, 1976, 1-90.

Goldman, P. S.: The role of experience in recovery of function following orbital frontal lesions in monkeys. Neuropsychologia 14: 401-412, 1976.

Goldman, P. S. and Alexander, G. E.: Maturation of prefrontal cortex in the monkey revealed by local reversible cryogenic depression. Nature 267: 613-615, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00481-06 LN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Neuroanatomical correlates of development and plasticity in primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Research Physiologist	LN NIMH
	H. E. Rosvold	Chief, Laboratory of Neuropsychology	LN NIMH
OTHER:	D. Tanaka	Assistant Professor	Howard University
	W. J. H. Nauta	Institute Professor	M.I.T.

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Massachusetts Institute of Technology

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6	1.8	4.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) To describe the maturation of cortico-cortical, commissural and cortico-subcortical connections in the developing primate; (2) To search for evidence of anomalous anatomical pathways resulting from early brain injury that would be capable of mediating behavioral recovery in the primate.

(1) Normal monkeys of various ages extending from birth through adulthood are given discrete unilateral lesions or unilateral injections of tritiated amino acids into the various areas of the cerebral cortex and sacrificed at appropriate intervals of time following surgery. Their brains are subsequently processed for anatomical analysis by silver impregnation or autoradiographic techniques.

(2) Monkeys that received bilateral orbital prefrontal lesions as infants and have demonstrated recovery of function at two years of age will be given unilateral lesions or injections of tritiated amino-acids in various regions of the brain hypothesized to be involved in mediating the recovery.

These studies should provide much needed information on the maturation and plasticity of specific neurobehavioral systems in the infrahuman primate.

1. Project Description:

The objectives of this research are twofold: (1) to describe the postnatal development of cortico-cortical commissural and cortico-subcortical connections in the primate; and (2) to search for evidence of anomalous pathways in the brains of monkeys exhibiting behavioral recovery following early brain lesions. The work reported here represents the preliminary phases of a major effort in the neuroanatomy of the developing brain. This year has been spent training staff, developing procedures and establishing laboratory facilities to accommodate a change in emphasis and orientation. It is too soon to expect major results and the present report is therefore identical to that of last year.

Monkeys of various ages extending from birth through adulthood are given discrete unilateral lesions or unilateral injections of tritiated amino acids into the various areas of the cerebral cortex and sacrificed at appropriate intervals of time following surgery. Their brains are subsequently processed for anatomical analysis by silver impregnation or autoradiographic techniques. Our studies using silver impregnation techniques have confirmed findings obtained with behavioral methods by indicating considerable immaturity in the argyrophilic reactions of pathways emanating from the orbital and dorsolateral prefrontal regions of the cortex to the caudate nucleus and other structures in monkeys under six months of age. The parallel autoradiographic studies now in progress have clarified and extended these findings. Specific findings include: (1) The cortico-cortical and commissural efferents of the frontal association and motor cortex terminate in a distinct columnar pattern. Since, until now, columns have been observed exclusively in sensory cortex, our findings in association and motor cortex indicate that columnar organization is a general feature of the mammalian neocortex. (2) Prefrontal cortico-cortical and commissural efferents terminate in all layers of target cortex, a feature of laminar distribution which distinguishes the frontal association cortex from sensory cortical areas. (3) Prefrontal cortex projects heavily upon the caudate nucleus whereas connections from motor cortex are sparse. This finding provides direct evidence that the caudate nucleus is related both anatomically and functionally more to the prefrontal cortex than to the motor cortex, and necessitates a revision of long-held conceptions about caudate function. (4) Specific regions of the prefrontal cortex project to the tail and body of the caudate nucleus as well as to its head. The distribution prefrontal-striatal connections revealed by autoradiographic methods changes the picture of topographic relationships between the cerebral cortex and the caudate based on silver degeneration methods. (5) Prefrontal projections to the caudate nucleus are stereometric, indicating that segregation of afferents applies to subcortical as well as to cortical structures and is a general feature of central nervous system organization. (6) Columnar organization in frontal cortex and stereometric

innervation of the caudate nucleus are well-delineated in the newborn monkey. The same features are present but not as pronounced in older monkeys given equivalent doses of radioactive amino acids. The differences between immature and mature monkeys may relate to a number of factors including developmental differences in axonal transport mechanisms, packing density of neurons and blood-brain barrier. These studies provide important new information on the development of circuitry, a parameter of neural maturation that is presumptively highly relevant to the development of behavioral capacity.

Studies of Central Nervous System Reorganization. Although central nervous system plasticity is presently an area of active research interest, there has so far been no compelling evidence to indicate that postlesion axonal growth, or collateral sprouting is functionally adaptive. Our previous studies of behavioral recovery in monkeys sustaining orbital prefrontal lesions in infancy show that the monkey is an excellent model of behavioral plasticity in development and one highly suitable for studies of neural reorganization. We are therefore employing both the Fink-Heimer silver impregnation technique and autoradiographic methods to examine the brains of monkeys, now adults, that received bilateral orbital prefrontal lesions as infants for evidence of anomalous connections. The brains of these monkeys are to be compared with those of monkeys that received identical lesions and survival times as adults and with normal controls. We have already obtained evidence both in silver-impregnated and autoradiographic material of a previously unknown pathway from the mid-region of the principal sulcus to the septum, a limbic system structure which is interconnected with the hippocampus and hypothalamus and which itself exhibits considerable potential for reorganization after partial deafferentation of its cortical input in rodents. It is too early to say whether a similar reorganization can take place after cortical deafferentation in primates. These longitudinal studies, which are still in progress, should provide important information on whether behavioral recovery following early brain injury can be attributed to the capacity of the developing nervous system for postlesion axonal growth.

2. Publications:

Goldman, P. S. and Nauta, W. J. H.: Autoradiographic demonstration of a projection from prefrontal association cortex to superior colliculus in the rhesus monkey. Brain Research 116: 145-149, 1976.

Goldman, P. S., and Nauta, W. J. H.: An intricately patterned prefronto-caudate projection in the rhesus monkey. J. Comp. Neurol. 171: 369-386,1977.

Goldman, P. S., and Nauta, W. J. H.: Columnar distribution of cortico-cortical fibers in the frontal association, limbic and motor cortex of the developing rhesus monkey. Brain Research 122: 393-413, 1977.

Johnson, T. N., Rosvold, H. E., Galkin, T. W. and Goldman, P. S.: Postnatal maturation of subcortical projections from the prefrontal cortex in the rhesus monkey. J. Comp. Neurol. 166: 427-444, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00490-01 LN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Single unit analysis of inferior temporal cortex in behaving monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. D. Jarvis	Research Psychologist	LN NIMH
OTHER:	M. Mishkin	Research Physiologist	LN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This has been incorporated into a new project Z01 MH 02032-01 LN.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02031-01 LN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in spatial perception

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mortimer Mishkin	Research Physiologist	LN NIMH
OTHER:	L. Ungerleider	NEI Extramural Fellow	LN NIMH
	F. J. Manning	Chief, Dept. Exp. Psychol.	WRAIR

COOPERATING UNITS (if any)

Walter Reed Army Institute of Research

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.4	0.6

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate and prestriate cortex project not only ventrally, to the inferior temporal area, but also dorsally, to the posterior parietal and dorsal prefrontal areas. This dorsal projection pathway appears to constitute the visual contribution to a system for spatial perception. Many modalities undoubtedly contribute to this spatial system, which can be divided into a posterior (parietal) portion serving allocentric localization, and an anterior (prefrontal) portion serving autocentric localization. The entire system is probably critical for the spatial guidance of movement, and in keeping with this its major cortical outflow appears to be to the motor system.

PHS-6040
(Rev. 10-76)

1. Project Description:

The two sub-projects described below deal with the parietal and prefrontal portions of the spatial system, respectively. (1) Animals trained on a visual landmark test are prepared with posterior parietal removals in one hemisphere and complete striate removals in the other, and these are then followed by callosal transection. Preliminary results indicate that the crossed lesions alone produce severe impairment, and that once the animal recovers the addition of a callosal transection has no effect. The results suggest that the posterior parietal cortex, like the inferior temporal, depends heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive bilateral visual input. Thus each posterior parietal area may be organized largely as a substrate for contralateral spatial function. (2) A crossed-lesion experiment like that described above has shown that the dorsal prefrontal cortex also depends on striate input for its visual function, in this case, autocentric localization as measured by the spatial delayed response test. Evidence that the function is selectively spatial comes from a recent experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests, including delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the principal sulcus quickly relearned, in sharp contrast to their performance on spatial memory tests.

Significance to mental health. Spatial orientation in animals is an extremely well developed, higher order, perceptual mode. It is "higher order" in the sense that it depends not on a single sensory modality but on the coordinated functioning of several of them. Our evidence points to a dual cortical localization for this suprasensory modality in the posterior parietal and dorsal prefrontal areas. Analysis of this complex, multimodal, cognitive system in monkeys may help us to understand the organization of yet another complex, multimodal, cognitive system in man, namely, the one for language. The formal similarities between the language system (for which man's left hemisphere is specialized) and the spatial system (for which his right hemisphere is specialized) give added support to the validity of this approach.

2. Publications:

Mishkin, M., Pohl, W., and Rosenkilde, C. E.: Kinesthetic discrimination after prefrontal lesions in monkeys. Brain Research 130: 163-168 (1977)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 02032-01 LN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neural Coding of Visual Stimuli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. D. Jarvis	Research Psychologist	LN NIMH
OTHER:	M. Mishkin	Research Physiologist	LN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.2	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our previous work has shown that cells in the inferior temporal cortex of awake monkeys can be driven selectively by stationary visual patterns and colors that the monkeys are required to discriminate. In the present study we asked whether the responses of these cells could be modified by reversing the motivational significance of the stimuli. In the reversal learning paradigm, stimuli originally associated with reward become negative, while originally negative stimuli become positive. Of the cells studied during a shift in the reward value of stimuli, and the accompanying shift in response requirements, the majority showed no changes in their pattern of firing in the two conditions. Of the cells that did show changes, only a few seemed to be sensitive to shifts in stimulus-reward association per se. The others were excluded from this category either because they showed changes in spontaneous discharges rather than in stimulus-locked activity, or because their changes in stimulus-locked activity appeared to reflect differences in the duration of fixation of the same stimulus in the two reward conditions. The data suggest that

inferior temporal neurons are more concerned with the physical properties of stimuli than with their motivational significance. By implication, the visual impairment after inferior temporal lesions is more likely to reflect a disorder in stimulus processing than in stimulus-reward association.

1. Project Description:

The importance of the inferior temporal cortex in vision has been demonstrated using both the lesion technique and the techniques of electrophysiology. The initial electrophysiological studies done with the anesthetized, paralyzed monkey, demonstrated that cells in this preparation were responsive to an array of stimuli over a large portion of the visual field, these large receptive fields always including the center of gaze. Using this information, we studied the responsiveness of cells to stationary stimuli in the behaving monkey, while the animal performed on an automated visual discrimination task.

Using a task in which each cell could be investigated with as many as 25 different patterns or colors, we discovered that the pattern of firing changed for about 40% of these cells on presentation of one or more of these stimuli. Most of the responsive cells were sensitive to an array of the stimuli and each of these cells had a rather unique stimulus profile. These results indicate that there is not a one-to-one correspondence between a particular stimulus and the firing of a particular cell, since a cell responded to many stimuli and a single stimulus evoked responses from many cells.

Once we had determined that IT cells could be driven by these stationary patterns, we next determined the effect on cell firing of changing the motivational significance of the effective stimulus. This change in the motivational significance was accomplished by using a reversal learning paradigm in which an originally positive simulus (one associated with reward) became negative and an originally negative stimulus became positive.

Eighty-percent of the nearly 100 cells studied with this paradigm continued to respond to effective stimuli in the same fashion, irrespective of changes in the motivational significance of the stimuli and associated changes in response requirements to those stimuli. Some of the changes in firing that did occur during shifts in reward value seemed related to differences in duration of fixation of the stimulus in the two reward conditions. Only a few of the cells seemed to be sensitive to shifts in stimulus-reward association per se.

The results of these two experiments suggest that processing visual information is the task of the IT cortex and that this cortex is probably not the site where visual stimuli are invested with motivational significance.

Directions of Future Research. A central question that remains unanswered by these initial studies is that of stimulus coding - to what characteristics of the visual world are cells in the IT cortex particularly sensitive? An answer to this question requires the kind of stimulus control that can be provided only by computer-generated displays which would allow manipulation of the stimulus in an effort to find the "best" one for the cell. Consequently, these critical studies must be postponed until the laboratory can be equipped with a computer and display device. Meanwhile, we have decided to approach the question of stimulus coding in another way. Recent studies have suggested that pattern vision may rest on the ability of the visual system to analyze objects in terms of their component spatial frequencies - i.e. the visual system appears to be sensitive to the spacing between light and dark areas and to differences in contrast (brightness) between these areas.

Our plan, therefore, is to study the contrast sensitivity of normal and brain-damaged monkeys to stimuli of different spatial frequencies using sine-wave gratings (bars in which the transition from light to dark is gradual rather than abrupt). Our hypothesis is that the ability to detect the presence of closely-spaced (high spatial frequency) bars over changing levels of contrast depends on the striate cortex, since good visual acuity depends on this cortex. On the other hand, the ability to detect the presence of widely-spaced bars (low spatial frequency) over changing levels of contrast may depend especially on the IT cortex, known to be important for pattern or configuration vision which does not depend on visual acuity.

If these lesion studies reveal differential sensitivities to spatial frequencies, we will use that information to study single unit response not only to different gratings, but to patterns which are similar to each other in their spatial frequency components. Perhaps then we will have an answer to why physically dissimilar stimuli evoke similar responses from a single unit.

There are several other lines of research which we plan to undertake concurrently with the spatial frequency project which are an outgrowth of both the preliminary single unit studies and recent lesion results.

In the normal animal, a visual discrimination learned with one eye transfers easily to the untrained eye. This interocular transfer is possible because information processed through the hemiretinae of the seeing eye converges on the same striate cortex cells which receive information from corresponding points on the hemiretinae of the other eye. In the absence of this binocular convergence via the optic chiasm, the forebrain commissures provide a converging input from non-corresponding points of the hemiretinae of both eyes onto inferior temporal neurons. Indeed, in the absence of the optic chiasm, the forebrain commissures have been shown to permit interocular transfer.

At the single unit level we would expect, therefore, that in the chiasm-sectioned monkey the stimuli capable of driving a cell through one eye would be the same stimuli effective in driving the cell through the other eye. Such convergence at the single cell level would offer support for the notion that the inferior temporal cortex is important in providing stimulus equivalence across the retinae - i.e. that a stimulus seen with one hemiretina impinges upon the same cortical elements that that stimulus impinges on when viewed with the opposite hemiretina. Without such a stimulus equivalence mechanism, we conjecture that objects would be recognized only if they fell repeatedly on the same retinal elements.

In the same animals in whom the question of stimulus convergence will be studied our plan is to investigate the possibility that a cell's response to a particular stimulus is a function of the ease with which that stimulus is discriminated from another with which it is being paired. To answer this question, the response of a unit to, for example, a vertically oriented bar will be measured when the stimulus with which it is paired is a ring and when the stimulus with which it is paired is a bar 15^{o} off the vertical. In the latter pairing the more difficult discrimination presumably requires greater attention to the absolute verticality of the stimulus. It is just such failure to notice or attend to the details of visual stimuli which has been invoked to account for the poor relearning of monkeys following posterior IT removals. Will differences in demands on attention be reflected in differences in the responsiveness of the cell in the two task situations?

Finally, there is another line of investigation we hope to pursue which also follows from the preliminary single unit studies. We have results, as indicated, suggesting that the IT cortex is probably not the site in which visual stimuli become invested with motivational significance. Recent results in this laboratory have indicated instead that the locus of such stimulus-affect association may be in structures to which the IT cortex projects, principally the amygdala. Using the same task that we employed to investigate stimulus-reward associations in the IT cortex, we will look at the activity of single cells in that part of the amygdala which has been identified as the target of IT output. In this area we might expect to find a high proportion of cells whose response characteristics change with shifts in the reward value of the stimulus.

Scientific Significance to Mental Health Research. It seems clear from both human and animal studies that the inferior temporal cortex in some way provides the neural substrate supporting visual perception, visual learning and memory, and visual attention. In the single cell studies that have been described, our goal is to discover the attributes of the visual world that determine the firing of the units in this cortex and, further, to discover the influence of cognitive factors such as attention and intention

to remember on firing characteristics. If we can understand the influence of both these physical and non-physical variables on the firing of cells in the normal brain, we may then have a clue to understanding aberrant perceptual and cognitive processes in various mental and nervous diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00471-22 LPP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of Heredity and Environment in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Rosenthal	Chief, Lab. of Psychology and Psychopathology	LPP NIMH
OTHER:	P. H. Wender	Professor of Psychiatry	University of Utah
	S. S. Kety	Professor of Psychiatry	Harvard Medical School
	S. Nagler	Research Psychologist	Institute for Research on Kibbutz Education (Israel)
	F. Schulsinger	Co-Director	Psychological Institute (Denmark)
	O. W. Quinn	Guest Worker	LPP NIMH

COOPERATING UNITS (if any)
Harvard Medical School, Institute for Research on Kibbutz Education, and Psychological Institute

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	3.1	2.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

PHS-6040

Project Description:

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

The analysis of the literature has been reported in a series of papers and in the study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders and a modified version of this book have been published. Several papers on our adoption studies are now published. The studies indicate that hereditary factors contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation, and eventually a monograph will be written on the adoption studies. Data analysis is continuing. One hundred subjects in the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs in childhood than do controls. Four subjects have had a breakdown and all have a schizophrenic parent.

It is significant to bio-medical research and the program of the Institute that we have resolved a chronic, critical problem in psychiatry by demonstrating beyond any reasonable doubt that genetic factors are importantly involved in the etiology of schizophrenia and related mental disorders. Moreover, the evidence thus far suggests that the mode of genetic transmission is polygenic or a dominant single gene with polygenic modifiers.

The proposed course of our project is to examine additional subjects in Denmark with the following goals in mind: (1) to try to discriminate gene carriers and non-gene carriers with respect to personality and test variables; (2) to compare the fate of gene carriers who are reared in the parental home and compared to those reared in adoptive homes; (3) to compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; (4) to find the incidence of schizophrenia spectrum disorder in a random sample of the Danish population. In Israel, our goal is to prepare a volume on our findings and their theoretical implications. During this year we are carrying out reexaminations of this unique sample of subjects.

Publications:

Kety, S.S., Rosenthal, D., Wender, P.H., and Schulsinger, F.: Studies based on a total sample of adopted individuals and their relatives: why they were necessary, what they demonstrated and failed to demonstrate. Schizophrenia Bulletin 2(3): U.S. DHEW, PHS, ADAMHA, NIMH. DHEW Publ. No. (ADM) 77-145. Washington, D.C., U.S. Govt. Print. Off., 413-428, 1976.

Mirdal, G.M., Rosenthal, D., Wender, P.H., and Schulsinger, F.: Perinatal complications in offspring of psychotic parents. Br. J. Psychiatry 130: 495-505, 1977.

Rosenthal, D. Searches for the mode of genetic transmission in schizophrenia: reflections and loose ends. Schizophrenia Bulletin 3(2): U.S. DHEW, PHS, ADAMHA, NIMH. DHEW Publ. No. (ADM) 77-145. Washington, D.C. U.S. Govt. Print. Off., 268-276, 1977.

Rosenthal, D. and Quinn, O.W.: Quadruplet hallucinations: phenotypic variations of a schizophrenic genotype. Arch. Gen. Psychiatry 34(7): 817-827, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00472-14 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Investigation of Some Formal Characteristics of Speech

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.S. Boomer	Research Psychologist	LPP, NIMH
OTHER:	T.P. Zahn	Research Psychologist	LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology & Psychopathology

SECTION

-

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	.8	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project investigates some physiological correlates of attention during natural speech perception. Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention, and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of GSR and reaction time to irrelevant stimuli--clicks--while the subject is listening to a tape recorded dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. If the fundamental hypothesis can be given empirical support this method will be used to investigate attention deficit in schizophrenia.

PHS-6040

Project Description:

An extended psycholinguistic research program, detailed in previous annual reports, has contributed to a developing theory of speech production and perception, original in this laboratory. The central hypothesis is that the phonemic clause, a linguistically defined unit of speech averaging about 7 or 8 syllables, is a strategic functional unit in both the production and perception of speech. The major findings from this project, previously reported, include demonstrations that speech non-fluencies such as hesitations, repetitions, and tongue-slips are not random noise but occur lawfully with respect to the structure of the phonemic clause.

More salient to our current work is a series of behavioral studies conducted by a former member of this laboratory, showing that the phonemic clause is also instrumental in the process of speech perception. He found that in telephone conversations and in face-to-face dialogue "listener responses," headnods and "mhms," are emitted by listeners predominantly at phonemic clause terminal boundaries.

During the past year the present research program has focused on speech perception. The guiding hypothesis, growing out of the work on listener responses, is that listeners, like speakers, process speech in "chunks," or temporal patterns, the chunks being phonemic clauses. If the decoding process operates with phonemic clause speech units then attention to the incoming speech need not be continuous, even though the speech itself is continuous. Rather than attending to fine detail in the stream of speech, syllable by syllable and word by word, the listener can accumulate a series of 7 or 8 syllables in short-term memory and at its conclusion decode it as a pattern of sound and meaning. As George Miller has suggested, "chunking" is an efficient perceptual strategy where central processing time is much faster than the rate of incoming information. If speech perception in fact operates in this way one would expect that the listener's attention would fluctuate, being maximally occupied at the end of the phonemic clause and minimally occupied while the clause is being accumulated and stored.

Preliminary tests of this hypothesis, undertaken last year, employed the cortical average evoked response as a measure of attention. We have shelved this approach, at least for the present, and have turned to reaction time as a dependent variable.

In the present study subjects listen to a rather absorbing tape recorded dialogue in one ear while also attending to an irregular series of clicks presented to the other ear. They respond to each click by depressing a telegraph key as quickly as possible. Reaction times are measured and stored. Half of the clicks are precisely timed to occur at the end, or terminal juncture, of phonemic clauses; the other half occur within phonemic clauses. Our hypothesis is that the subjects' attention to the cognitive demands of speech decoding will be different during clauses than at the ends of completed clauses, and this difference will be reflected in the mean response latency to the two sets of linguistically-specified clicks.

Beyond the present normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular attention to the specification of hemispheric asymmetry in cortical function. It is widely believed that cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically the ability to extract the salient elements of complex stimulus patterns. Since speech is just such a complex pattern and since the hemispheres are asymmetrically specialized for speech, a psycholinguistic approach to the problem of laterality and attention seems to be both neglected and promising.

Publications:

Boomer, D.S.: Speech Units in Human Communication. In Siegman, A.W. and Feldstein, S. (Eds.): Nonverbal Behavior and Communication. New Jersey, Lawrence Erlbaum Associates. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00473-20 LPP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Individual differences in normal perceptual processes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: V. R. Carlson Research Psychologist LPP NIMH
Other: None

COOPERATING UNITS (if any)
I. Feinberg, Veterans Administration Hospital, San Francisco
K. L. Norman, Department of Psychology, University of Maryland

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 2001

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The assessment of perceptual capacities generally requires measurement of the subject's ability to integrate information from more than one stimulus dimension. Judgments of this kind are susceptible to various biases arising from cognitive, motivational, and other context effects, often invalidating the measurement. A general methodology, known as functional measurement theory, is being developed to deal with this problem in behavioral information processing. A study is under way to evaluate the usefulness of the functional measurement approach in assessing perceptual constancy judgments. These judgments involve the integration of size and distance information and are known to be affected by attitudinal biases. The question to be answered is whether the functional measurement procedure will result in reliable and valid estimates of perceptual ability, particularly for individual subjects. The data are currently being collected.

Project Description:

The general objectives are to develop valid methods for the assessment of perceptual functioning and to obtain comparison data for evaluating the effects of abnormal and other special conditions. A study has been initiated to investigate the usefulness of functional measurement theory in assessing perceptual constancy.

Functional measurement theory is a currently developing general methodology for dealing with problems in multidimensional information integration. Perceptual constancy requires the integration of stimulus information such as size, shape, and distance into a unitary percept, and usual measures of the ability to do this are known to be affected greatly by attitudinal biases. A major biasing effect can be demonstrated by varying instructions to subjects to judge apparent visual magnitudes or objective magnitudes. The purpose of this study is to determine whether reliable and valid estimates of perceptual ability can be obtained, in spite of the known biasing effects, by applying the techniques of functional measurement.

Functional measurement tests the validity of measurement by evaluating logically and mathematically required consistencies in judgment for different combinations of stimuli. In the present experiment subjects are presented with two triangles and required to adjust the size of a third triangle to the average of the first two. Sixteen size combinations are presented at nine different distance combinations. Subjects make the judgments under instructions to match apparent visual size or to match objective, physical size. The criteria of consistency will be applied both to the averages of the several experimental groups and to the data of individual subjects. Data are currently being collected on normal young adults.

In studying behavioral effects associated with pathology or drug conditions or in investigating the neurophysiological or biochemical processes underlying behavioral effects, it is as essential to achieve scientifically valid measurement of the behavioral variables involved as it is to measure the non-behavioral variables adequately. Functional measurement theory is potentially wide in scope, applicable in principle to any behavioral assessment of perceptual-cognitive information integration.

Publications:

Carlson, V.R.: How Are You Coming Through? In Ferry, T.S. and Weaver, D.A. (Eds.): Directions in Safety. Springfield, Ill., Charles C. Thomas, 1976, pp. 437-446.

Carlson, V.R. and Feinberg, I.: Judgments of short time intervals following awakenings from different EEG stages of sleep. Physiol. Psychol. 4: 341-345, 1976.

Carlson, V.R.: Instructions and Perceptual Constancy Judgments. In Epstein, W. (Ed.): Stability and Constancy in Visual Perception. New York, John Wiley & Sons, 1977, pp. 217-254.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00474-17 LPP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Perceptual adaptation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: V. R. Carlson Research Psychologist LPP NIMH
OTHER: None

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Psychology & Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was reported last year as inactive, and we wish to discontinue it.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00476-06 LPP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Offspring of Schizophrenics: Markers of a Schizophrenic Disposition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP, NIMH
OTHER:	D. Rosenthal	Research Psychologist	LPP, NIMH
	S. Broman	Research Psychologist	DNB, NINCDS
	P. Nichols	Research Psychologist	DNB, NINCDS
	P.H. Wender	Professor of Psychiatry	Univ. of Utah

COOPERATING UNITS (if any)
Developmental Neurology Branch, NINCDS

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
-

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Marlyand 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.6	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of children of schizophrenics is based on data from an NINCDS study of pregnancy and delivery conditions in a large group of women, which included psychological and neurological tests on the offspring, studied until age 8. We have found that: (1) There were more fetal deaths among the offspring of schizophrenics. (2) The IQ scores of the surviving offspring of schizophrenics appear to be more related to adverse perinatal factors than do IQ scores for the controls. (3) The behavior of the surviving male offspring of schizophrenics was more often rated as hyperactive or immature at age 7 than was the behavior of controls. These offspring also had more soft neurological signs, especially abnormal hand-arm coordination.

PHS-6040
(Rev. 10-76)

Project Description:

The project is aimed at discovering differences between the offspring of schizophrenics and controls, at an early stage in life, which may represent a predisposition to develop schizophrenia later in life. It is also aimed at evaluating the effect of perinatal complications on the children of schizophrenics. This study has utilized the data previously collected by the National Institute of Neurological and Communicative Disorders and Stroke, Perinatal Research Branch, in their "Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood." In that project, some 55,000 pregnancies and the offspring were studied, with the 8-year follow-up now just completed. About 900 of the mothers or fathers had had a psychiatric hospitalization, and of this group, we took 220 who were studied in the Boston area to use for our study. We gathered the records of their psychiatric hospitalizations, and we proceeded to diagnose these subjects. We diagnosed 150 parents, and assigned a diagnosis of schizophrenia to 67 of these. The 150 families produced a total of 210 children who had been part of the NINCDS study, and 93 of these were the offspring of schizophrenics. Taking the families containing a schizophrenic parent, we selected a matched control group, and began comparing the findings for our two groups on the investigations which the NINCDS/PRB had performed.

We have found that there is an increased rate of fetal and neonatal death among the offspring of schizophrenics as compared with controls. Some of these deaths were associated with neurological malformations. Reasons for the deaths were not found at autopsy. The deaths were among the offspring of parents with chronic and severe forms of schizophrenia, as opposed to the acute or borderline forms. The deaths occurred predominately among those offspring who had two schizophrenic parents, or had a schizophrenic mother, rather than among those who had a schizophrenic father only. Two comparison groups, offspring of possible schizophrenics and offspring of nonschizophrenic patients, did not show an increased rate of fetal or neonatal deaths when compared to their matched controls.

There is a slightly lower IQ among the offspring of parents with the more chronic forms of schizophrenia when compared with controls at 7 years. Perinatal factors, such as edema, vaginal bleeding, spinal anesthesia at delivery and Apgar Score are quite highly correlated (in a negative direction) with IQ in this sample, whereas they are not highly correlated among the control population. These negative correlations were present whether the father or the mother was the schizophrenic parent, and thus the findings might reflect a genetic susceptibility of the infant to perinatal complications. By means of a multiple regression analysis it was discovered that these perinatal factor-IQ correlations could not be accounted for by demographic variables such as SES, maternal age, parity, etc.

Certain behavioral abnormalities, namely hyperactivity and immaturity (shyness emotional reactivity) have been frequently found among the male offspring of parents with the more chronic forms of schizophrenia. It does not necessarily follow that these behavioral disorders are genetically determined, but it is quite possible that the children with these abnormalities are the children who will later become schizophrenic, since both hyperactivity and immaturity

are frequently found in the childhood histories of adult male schizophrenics. We plan to investigate the perinatal factors which correlated with these behavioral abnormalities, as we did with the offsprings' IQ.

The significance of this work to bio-medical research and the program of the Institute is that it adds to our knowledge of the etiology of schizophrenia. We need to find how the genetic factor underlying schizophrenia clinically manifests itself if we are to do biochemical and physiological investigations of the development of schizophrenia or to effectively intervene. If a susceptibility to certain perinatal complications is demonstrated, it would direct research and intervention to the perinatal period.

This project will be discontinued after the analysis of the behavioral abnormalities in the children has been completed.

Publications:

Rieder, R.O., Broman, S.H., Rosenthal, D. The Offspring of Schizophrenics II: Perinatal Factors and IQ. Archives of General Psychiatry. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00484-17 LPP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Psychophysiological Responsivity and Behavior in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	C. Schooler	Research Psychologist	LSES, NIMH
	W.T. Carpenter, Jr.	Director of Sz. Research	Albert Einstein College
	T.H. McGlashan	Staff Psychiatrist	Chestnut Lodge
	M. Buchsbaum	Research Psychiatrist	APB, NIMH
	D. Murphy	Research Psychiatrist	LCS, NIMH
	D. Van Kammen	Research Psychiatrist	APB, NIMH
	P. Fedio	Research Psychologist	SNB, NINCDS

COOPERATING UNITS (if any)
Laboratory of Socio-environmental Studies, Adult Psychiatry Branch, Laboratory of Clinical Science, Surgical Neurology Branch and Ward 4 East

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
-

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.8	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

General purpose: To investigate schizophrenic-normal differences in autonomic nervous system activity in various laboratory situations which differ in the demands placed on the subject, the kinds of stimuli presented and the type of behavior required. To determine biological and psychological processes related to ANS activity.

Peripheral measures of autonomic functioning: Skin conductance, heart rate, finger pulse volume, respiration and skin temperature are recorded continuously during periods of resting, presentation of mild tones and task performance. The tasks used include reaction time, mental arithmetic, two-flash threshold, and tachistoscopic recognition.

We showed that drug free acute schizophrenics who did not improve during a subsequent 4 month hospitalization were more deviant in ANS functioning at admission than were patients who improved. Current studies aim to replicate and extend this finding in patients who are on controlled drug trials to see if on or off drug ANS activity is related to therapeutic outcome.

Project Description:

In a completed study 46 acute schizophrenic patients were tested 3-4 weeks after hospital admission and again 3-4 months later just before discharge, the patients being drug free on both occasions. Compared to 118 normal controls the patients had significantly higher autonomic arousal than the controls on several electrodermal and cardiac indices, but one commonly used index of arousal (skin conductance level) was in the opposite direction. The patients had less phasic ANS responsivity to a series of meaningless tones as well as to the more demanding stimuli of a reaction time task and lower tonic activation during a stress procedure (mental arithmetic test) particularly during the instructions. The schizophrenics also habituated more slowly.

Comparisons of patients who subsequently showed significant improvement at the end of the 4 month hospitalization with patients who were still acutely psychotic showed that despite equally severe psychopathology ratings at the admission testing the admission psychophysiology and attention of the subjects who improved was much more similar to normal than was that of the not-to-improve group. On almost all of the variables which distinguished the patient group as a whole from controls, the not-to-improve group was significantly more deviant from the controls. The tests at discharge showed surprisingly little change from the admission testing even in the patients who showed marked clinical improvement.

These results suggest that early improvement from an acute schizophrenic episode is predictable from ANS activity during the episode but not from the severity of psychopathology during the episode or from conventional prognostic indicators. The small changes in psychophysiology accompanying clinical improvement suggests either that these variables are persisting individual characteristics similar to traits or that "normalizing" of the ANS activity preceded clinical improvement. The patients have been evaluated and retested at a one year followup, and these observations will hopefully clarify the longer-term implications of these findings.

Two new experimental tasks are being used with both schizophrenic patients and normal controls. One is a two-flash threshold determination which purportedly is a measure of "cortical arousal" and which has been found to vary with autonomic arousal level in different directions for schizophrenic and normal subjects. The other is a tachistoscopic recognition procedure which lends itself to concurrent psychophysiological recording. With this technique we hope to be able to test the differential psychophysiological responsiveness of schizophrenics and normals to such things as task difficulty, success and failure, positive and negative reinforcements, etc., as well as being able to separate sensory reception aspects from response aspects of the stimulus complex.

Autonomic functioning has been recorded during performance of these tasks in two sessions which included rest periods and a stress period in which a balloon is blown up until it bursts. These procedures are being given to a

new sample of acute schizophrenic patients during a drug free period shortly after Clinical Center admission. In addition to attempting to replicate the previous findings on the relation of ANS activity to improvement we can also relate the ANS variables to the effects of different drugs such as lithium and pimozide which are being tried along with placebo on a double-blind basis on these patients. Thus, we can test the feasibility of using ANS variables to aid in the determination of the optimal therapy. In addition, we have begun testing patients while they are on the drug protocol using a procedure in which ANS variables are recorded during rest, presentation of a series of tones of different intensities and a two-flash threshold determination. These observations may help us determine how ANS changes relate to changes in behavior and to increase our understanding of the central mechanisms involved in of ANS functioning.

In two studies with normal subjects we are trying to learn more about the relationships between ANS activity, personality and behavior. About 90 normal subjects have been tested on the same procedures given to our current schizophrenic group at admission (which are described above), were given personality, interest, and mood questionnaires and had platelet and plasma monoamine oxidase activity measured. The data relating MAO to personality and interests confirm previous findings that low platelet MAO is related to "stimulus seeking," and suggest that high MAO is related to a rather lethargic approach to life. In general, MAO activity seems more related to an affective dimension in normals than to a schizoid dimension. Preliminary analyses of the autonomic data show that in women high ANS activity is related to high MAO activity and with low stimulus seeking behavior. In men these relationships are less pronounced, but high ANS activity is characteristic of men with active life styles. Analysis of the data is continuing. A similar study emphasizing autonomic orienting responses to tones of different intensities and using an overlapping battery of personality scales is in progress in collaboration with Dr. T. Robinson.

An investigation of the relationships between the ANS response to stress and changes in peripheral catecholamines is being started in collaboration with LCS and APB. Subjects, including hypertensives, will be given 3 psychological stress procedures (having a balloon popped, mental arithmetic, and electric shocks) interspersed with rest periods. Skin conductance and heart rate will be recorded continuously, blood pressure will be taken frequently, and periodic blood samples will be drawn and analyzed for catecholamines and for dopamine-beta-hydroxylase. Hypertensives will be compared with controls on all variables and changes in peripheral ANS activity will be correlated with changes in catecholamine levels.

Publications:

Zahn, T.P.: On the bimodality of the distribution of electrodermal orienting responses in schizophrenic patients. J. Nerv. Ment. Dis. 162: 195-199, 1976.

Zahn, T.P. & Carpenter, W.T. Effects of short term outcome and clinical improvement on reaction time in acute schizophrenic. J. Psychiatric Research, in Press.

Zahn, T.P. Autonomic nervous system characteristics possibly related to a genetic predisposition to schizophrenia. Schizophrenia Bulletin 3: 49-60, 1977.

Zahn, T.P.: Comments on "Reaction time and Attention in Schizophrenia." Schizophrenia Bulletin, in Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00485-05 LPP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Psychophysiological Changes During the Menstrual Cycle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: T.P. Zahn Research Psychologist LPP, NIMH
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
-

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was reported last year as temporarily inactive, and we wish to discontinue it.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00486-05 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Psychophysiological Concomitants of Minimal Brain Dysfunction in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	P.H. Wender	Professor of Psychiatry	University of Utah
	B.C. Little	Former Guest Worker	LPP, NIMH
	J.L. Rapoport	Medical Officer	BPB, NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

-

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many investigators believe that the autonomic nervous system may be involved in minimal brain dysfunction in children. The objectives of our studies are to investigate differences in autonomic functioning between MBD and normal children by means of peripheral indicators such as skin conductance, heart rate and pupillary activity, to predict which MBD children will respond favorably to stimulant drugs, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on attention such as reaction time. The hypothesis that MBD and normal children respond differently to stimulant drugs is being tested in a study of the effects of d-amphetamine on autonomic activity and attention in 6-13 year-old normal and MBD boys.

PHS-6040
(Rev. 10-76)

Project Description:

In our previous studies we did not find lower baseline autonomic activation in the MBD children when off drugs as some (but by no means all) investigators have claimed. In fact, the MBD group had some higher than normal indices of arousal in one session where the amount of environmental stimulation was relatively high. The MBD group, however, showed diminished and "sluggish" responsivity to stimuli on several autonomic measures: skin conductance, heart rate, and pupil size. They also were slower in reaction time performance which indicates a deficit in attention. Drugs improved attention and increased baseline activation levels but did not clearly affect autonomic responsivity. The hypothesis that autonomic variables might predict clinical response to stimulant drugs was not definitely testable because of the very few subjects that failed to respond clinically.

In a current study in collaboration with Dr. J.L. Rapoport, 6-13 year old normal boys and boys with a diagnosis of hyperactivity are being tested on attention (reaction time) and autonomic reactivity (skin conductance and heart rate) after being administered either placebo or d-amphetamine (.5 mg/kg) in a double-blind crossover study. The procedures used are very similar to those used in a previous study with a larger sample of hyperactive children. The purpose of this study is to test the widely held hypothesis that hyperactive children give paradoxical or atypical responses to stimulant drugs.

Publications:

Zahn, T.P., Little, B.A. & Wender, P.H. Pupillary and heart rate reactivity in children with minimal brain dysfunction. Journal of Abnormal Child Psychology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00487-05 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Autonomic Functioning in MZ and DZ Twins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	M. Buchsbaum	Research Psychiatrist	APB, NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

-

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine the relative importance of genetic vs. environmental factors in various aspects of autonomic functioning and to determine the relationships of autonomic nervous system activity to personality, task performance and electrocortical functioning.

Peripheral measures of autonomic functioning, namely skin resistance, heart rate, finger pulse volume, respiration, and skin temperature are recorded during two sessions which include rest periods, a series of mild tones, a reaction time task and mental arithmetic. The subjects are 30 MZ and DZ pairs equally male and female. Some opposite sex DZ pairs have also been tested.

PHS-6040
(Rev. 10-76)

Project Description:

A subgroup of these subjects has been used as the normal control group for the schizophrenics on Project Z01 MH 00484-16. Intraclass correlation will be done on base levels, responsivity to non-demanding and demanding stimuli, variability and response specificity. Correlation of autonomic data with personality, behavioral and electrocortical data on the same subjects will b done.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00488-12 LPP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Individual Differences in Survival and Reproduction Among Old Colony Mennonites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Allen	Research Geneticist	LPP NIMH
OTHER:	C. W. Redekop	Prof. of Sociology and Anthropology	Goshen College

COOPERATING UNITS (if any)
Goshen College

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This project is temporarily inactive.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00489-20 LPP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Variables Affecting Twin Birth Frequencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. Allen Research Geneticist LPP NIMH

COOPERATING UNITS (if any)

Perinatal Research Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Significant variations in the twinning rate can be observed both in the long term and in the short term. These may be explained under the hypothesis that women who bear dizygotic twins tend to conceive more readily than other women, and the difference in waiting time to conception has been estimated from national statistics to be about two months. If confirmed, the hypothesis would have implications for the physiology of reproduction.

Data on about 50,000 pregnancies collected prospectively in the Collaborative Perinatal Project will permit a direct test of the hypothesis and more precise estimation of any difference in average waiting time.

The mothers of dizygotic twins will be compared with matched controls with respect to fertility and birth intervals. This analysis is now approaching completion.

Project Description:

This project constitutes a new phase in a program of research begun in 1957. The earlier research led to the hypothesis that women who bear dizygotic twins are particularly fertile, a hypothesis reached independently by Finnish geneticists and supported by data from several sources. From U.S. birth statistics I was able to estimate the apparent difference in waiting time to conception, called "fecundability," between twin-prone women and other women.

If twin-prone women are more fertile than others, this fact, interacting with contraception, might explain the known variation in twin birth frequencies among countries and between years or decades. Before this can be attempted in detail, there is need for independent confirmation of the high fecundability of twin-prone women and a better quantitative estimate.

Nearly ideal material for these purposes was collected in the Collaborative Perinatal Project. The Perinatal Research Branch, NINCDS, has extracted from their file the relevant information on about 50,000 pregnancies, and this condensed file is now being analyzed.

If it can be established that twin-prone women become pregnant much more readily than other women, this may be of value to reproductive physiologists in isolating the variables that determine individual differences in ease of conception. It may also have more general significance, since approximately one-fourth of all women are twin-prone.

Findings:

Preliminary analysis tends to confirm the hypothesis that twin-prone women are more fertile than other women. The difference is seen as a 25% reduction in birth intervals and as a 12% increase in total children born. These estimates have large standard errors, however, and the final estimates may be somewhat larger after removal of a known source of systematic bias in the data.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00491-01 LPP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Personality factors and psychophysiological response to changing stimulus input.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas N. Robinson, Jr. Staff Fellow LPP NIMH
OTHER: Theodore P. Zahn Research Psychologist LPP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is designed to shed information on how such factors as stimulus intensity, unchanged stimulus input, heteromodal stimulation, and sensory threshold task performance differentially affect autonomic nervous system (ANS) functioning in selected personality groups. Examined also is the relative correspondence of bilateral electrodermal activity and its relation to perceptual task performance. Black and white males are sampled in an effort to provide data on racial differences in ANS functioning to imperative and uncontrolled stimulus input.

PHS-6040
(Rev. 10-76)

Project Description:

The objectives of this project are to provide further clarification of the relationship of psychometric personality factors and psychophysiological response in normals and to assess the extent to which racial groups show ANS response stereotypy.

Data collection for each subject comprises two sessions separated by at least a week. Each session involves approximately 2½ hours of testing. Fifty-four volunteers have been tested to this point. Data analysis for the first session is nearing completion in order to present preliminary findings at an upcoming research conference.

This project is significant to biomedical research and the program of the Institute in that a greater clarification of ANS functioning in normals will make more meaningful the comparison of normals and psychiatric patients in future psychiatric research. Moreover, because personality structure may play an important role in the expression of psychopathological illness, it is possible that a determination of the patterns of psychophysiological response associated with personality factors may help account for the large variability in ANS response noted among psychiatric patients. The determination of differential ANS response patterns for racial groups has implications for t genetics of psychophysiology and, as such, to the use of these techniques in discriminating aspects of the genetical component of mental illness.

The proposed course of the project is to continue testing until a sufficient sample size (e.g., 150) has been attained. We also anticipate expanding the project to include female subjects and psychiatric interview information on a selected number of individuals previously tested under the original protocol.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00492-01 LPP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Relationships among personality, biochemical and psychophysiological variables in college students.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Haier	Staff Fellow	LPP NIMH
Other:	M. S. Buchsbaum	Research Psychiatrist	BPB NIMH
	P. J. Khouri	Research Psychiatrist	LPP NIMH
	R. O. Rieder	Research Psychiatrist	LPP NIMH
	D. Murphy	Chief, Clin. Neuropharm.	LCS NIMH

COOPERATING UNITS (if any)
R. Coursey, Dept. of Psychology, University of Maryland

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

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1.0	.5	.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Four hundred college students completed the Minnesota Multiphasic Personality Inventory (MMPI). A subsample with scores suggestive of psychological difficulty also were tested for platelet monoamine oxidase (MAO) and for averaged evoked response (AER) patterning. Each of these Ss also was interviewed with a structured psychiatric interview. The purpose of the study is to establish the empirical relationships among MAO, AER, and clinical symptomology. The major hypothesis is that, as with chronic schizophrenia and affective disorders, low MAO and "augmenting" AER will predict clinical symptoms of psychological difficulty in a non-hospitalized population. No research results are available at this writing.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00493-01 LPP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of thought disturbance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Haier	Staff Fellow	LPP NIMH
Other:	D. S. Boomer	Research Psychologist	LPP NIMH
	H. Weingartner	Research Psychologist	LPP NIMH

COOPERATING UNITS (if any)

Ward 4E, BPB, NIMH

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Based on previous research in aphasia we have developed a simple psycholinguistic procedure to assess one aspect of thought disorder associated with schizophrenia. The procedure tests the strength of syntactic structure in sentence processing. We are testing the hypothesis that syntactic structure breaks down in schizophrenics when it is stressed with lexical associations. This hypothesis is important with respect to understanding the psychological nature of schizophrenia thought. No research results are available at this writing.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00494-01 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical and Empirical Detection of Simulated Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP, NIMH
OTHER:	D. Rosenthal	Research Psychologist	LPP, NIMH
	R. Haier	Research Psychologist	LPP, NIMH
	Paul Alexander	Clinical Associate	APB, NIMH
	Victor Reus	Clinical Associate	APB, NIMH

COOPERATING UNITS (if any)

St. Elizabeth's Hospital, Psychodrama Section

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project will investigate the ability of psychiatrists to differentiate schizophrenic patients from actors simulating schizophrenia using a 30 minute interview.

Videotapes will be made so that a number of psychiatrists may be used as raters.

The project is in progress and no data have yet been analyzed.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00495-01 LPP

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
The storage and retrieval of experience: psychological and biological determinants.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Weingartner	Research Psychologist	LPP NIMH
OTHER:	E. D. Caine	Staff Psychiatrist	BPB NIMH
	M. H. Ebert	Chief, Sec. Exper. Therapeutics	LCS NIMH
	R. Haier	Staff Fellow	LPP NIMH
	D. L. Murphy	Chief, Sec. Clin. Neuropharmacol.	LCS NIMH
	R. M. Post	Unit Chief, 3W	BPB NIMH
	J. L. Rapoport	Medical Officer	BPB NIMH
	D. P. van Kammen	Chief, Unit on Neuropsychopharm.	BPB NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, DCBR, NIMH
Laboratory of Clinical Science, DCBR, NIMH

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	.2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Research efforts have focused on the use of cognitive (information processing systems) strategies for exploring the mechanisms of thinking, learning, encoding and retrieval of experience in: mania, depression, the schizophrenias, the "organic" dementias (such as Huntington's Disease, Korsakoff's Psychosis, Alzheimer's Disease) as well as in drug altered brain states. Findings have helped clarify the determinants of encoding, memory storage and retrieval that characterize the cognitive changes in these different clinical syndromes. As such, it has helped to better define some of the psychological characteristics that are expressed in these altered brain state conditions and has tied some of these to underlying biological events. Data have emerged showing specific deficits in some components of information processing, changes in processing and retrieval strategies, state dependent (specific) learning and retrieval and changes in memory consolidation, all of which are drug and clinical state specific.

Project Description:

We have attempted to use the techniques and theory of contemporary information processing research (cognitive research) to 1) explore a number of clinical syndromes in man including depression, mania, schizophrenia, as well as some "organic" dementia's such as Alzheimer's Syndrome, Huntington's Disease and Korsakoff's Psychosis; 2) examine changes in cognitive processes that occur acutely in response to drug and behavioral manipulations of brain state in both patient populations and in normal controls; 3) define the psychological and biological determinants that might help account for the acquisition (learning) and later retrieval of experience in normal subjects as well as in patients functioning in some specifiable altered state condition. The underlying theme and approach to all of the studies completed to date, those in progress, and those presently being planned, contain the following two broad elements. First we have found that cognitive models are useful in helping to define altered brain states in man. Such an approach can provide a powerful leverage and increased clarity and focus for describing, in both a qualitative and quantitative manner, the component mechanisms that determine changes in information processing and learning in clinical syndromes in man. Secondly, it is clear that cognitive models are as yet quite incomplete and by using both clinical findings and those which emerge as cognitive responses to discrete drug alterations of brain state it is possible to build more viable models of cognitive processing in the normal information processor. Most cognitive theories to date have essentially ignored clinical findings, biological factors and data that have emerged from animal learning studies. Our research has been deliberately designed to bridge these ordinarily disparate areas of research.

The following areas of study are presently being pursued within this framework. It should be pointed out that, although these have been designed as independent studies, they are intimately related to one another and are constructed so as to fit within the broad framework defined above.

1. State dependent learning (SDL)

A number of drugs as well as some behavioral and clinical states appear to induce discrete changes in how events are encoded (e.g., transformed, organized, interpreted), and in a related manner, how events are remembered at some later point in time. Using an SDL framework we are examining how discrete changes in brain are expressed in qualitative and quantitative changes in information encoding, acquisition and retrieval. This research has been pursued in the following experiments:

a) Changes in mood state and SDL.
b) Cognitive SDL effects associated with the menstrual cycle and related biological effects.
c) Amphetamine, marijuana, alcohol, and physostigmine SDL response and related state dependent storage (SDS) and retrieval (SDR) of information.
d) Brain state arousal as a determinant of specific encodings and retrieval of events.
e) Mechanisms of SDL, SDS, and SDR in man and their implications for models of information storage and retrieval in man.

2. Mechanisms and strategies of information storage in the depressed state.

a) Brain state arousal, reinforcement and the temporary (short term memory) and permanent memory trace of experience.
b) Uncertainty and structure in the encoding of events.
c) Brain laterality and its expression in changes in mood and as a consequence of language vs. pattern processing, serial vs. parallel processing, gestalt vs. detailed processing.

3. Drug and behavioral studies of memory consolidation.

a) Physostigmine and choline post-learning treatment and the time x intensity (dose) post learning drug effect in forming a permanent trace of experience.
b) Stimulus characteristics that define various components of information storage and trace consolidation.
c) Post-learning arousal and reinforcement and its effects on recall of strong vs. weakly encoded events.
d) Sleep, specific sleep stages and sleep deprivation in determining what events will be effectively consolidated in memory.

4. The schizophrenic thought disorder.

a) The structure of encoded events and the recall of events as a model for defining the schizophrenic thought disorder.
b) Syntactic organization in schizophrenic thinking.

5. The structure, storage and retrieval of learning in normal and hyperactive children under stimulant drug and placebo conditions.

6. The mechanisms of learning and remembering in the "organic" dementias.

   a) Characterizing and contrasting the processes of storage and retrieval of information in Huntington's disease with that seen in Korsakoff's psychoses, Alzheimer's disease & Parkinson's disease. This research has both a clinical focus and attempts to explore some of the basic biological factors that account for discrete stages in information storage and retrieval by contrasting and relating different brain pathology with resulting discrete cognitive changes.
   b) Drug manipulations of demented patients and resultant specific changes in cognition.

Methods

The studies outlined above have been carried out using an extensive variety of learning procedures which have been specifically designed and developed by this investigator to explore focal issues that might relate cognition to specific aspects of brain state. These include:

   a) Strategies for manipulating the encoding of events. These techniques include the use of sorting procedures and accompanying uncertainty analysis (see Caine, Ebert, & Weingartner, in press; Weingartner, Caine, & Ebert, in press); manipulation of context at the time of storage and retrieval (Weingartner, in press; Weingartner & Murphy, 1977); systematic changes in the kinds of stimuli to be processed and their encodability (Weingartner, Adefris, Murphy & Eich, 1976; Weingartner & Murphy, in press); changes in the surrounding consequences or context for storing and retrieving events (Weingartner, Martello & Murphy).
   b) Immediate and delayed recall as well as analysis of input serial position in relation to output of information at the time of retrieval of information. These techniques have been used to explore state models of information processing and retrieval.
   c) A "levels of processing" approach to information storage and retrieval. The techniques embodied in this approach use methods for manipulating depth or salience of processing (through manipulations of arousal or orienting task) as a means of defining how effectively input information has been encoded. It also stresses distinctions in retrieval analysis between "availability" and "accessability" of learned events.

d) Free recall, serial learning, prompted recall, paired associates, cued recall, recognition memory, pattern learning, as well as other procedures have been designed for use in examining specific hypotheses that might capture cognitive changes.

Major Findings

State dependent learning. Mood state in man provides a psychological and biological context for uniquely encoding and retrieving information. During periods of both mania and depression experienced events are retrieved in a state specific manner (Weingartner, Miller, & Murphy, 1977). Information stored in one mood state is available but inaccessable (dissociated) in a disparate mood state (Weingartner, Martello, Murphy, in press). Meaningfully processed events are more susceptible to mood state specific memory dissociation than peripheral events. There are some preliminary findings that would indicate that this may, in part, be determined by state specific changes in brain laterality in information processing (Weingartner, Adefris, Eich, & Murphy, 1976; Weingartner & Murphy, in press).

We have also just demonstrated that the menstrual cycle also induces SDL, SDS, and SDR changes. These changes in dissociations in memory and learning are not, however, related to changes in mood but are correlated with changes in estrogen.

The effects of amphetamine, marijuana, physostigmine, and alcohol in inducing SDL, SDS, and SDR are continuing. We have found that these drug-induced dissociative effects can be altered by cueing, changing stimulus processing, and by relating events that are time tagged in episodic memory. SDL, SDS, and SDR can also be induced after learning has been established during a consolidation (rehearsal) phase of memory acquisition (Weingartner, in press). We are also exploring whether changes in mood are a necessary condition for the induction of state specific dissociations of memory (Stillman, Galanter, Lemberger, Fox, Weingartner, & Wyatt). Change in brain state arousal may be a specific biological determinant for providing a unique context for processing, encoding, and retrieving events. This finding is emerging from much of our recent explorations of drug and mood SDS, SDR research.

The mechanisms of SDL, SDS, & SDR have been increasingly useful in defining the psychological and biological factors that should be included in viable models of memory. Our SDL research has supported notions of memory and learning which intimately link and define what has been learned or stored in terms of the specificity of retrieval conditions, and that concepts of levels and kinds of information processing are more effective in describing the psychology of memory storage and retrieval (Craik,

Lockhardt, Jenkins, & Tulving) than are notions of discrete memory stages (Norman, & Wichelgren).

Mechanisms and strategies of information storage in the depressed state. Depressed patients have previously been shown to demonstrate specific deficits in long-term memory-learning-stor of information. These changes in memory-learning are directly correlated with the intensity of depression. These defects in cognition can also be reversed by some drugs that alter brain biogenic-amine activity. We have been able to show that post-learning behavioral arousal appears to have a pronounced effect on components of memory consolidation in normal subjects (Weingartner, Hall, Murphy, & Weinstein, 1976). We are presently planning to test whether such specific arousal in depressed subjects might alter the memory defect seen in depression.

We have found that by experimentally providing the experience of success (reinforcement) that memory-learning defects associated with the depressed state can be temporarily lifted (Weingartner, Martello, & Murphy, submitted for publication). These studies are also being pursued using drugs that might alter mood, REM sleep, and ultimately, information consolidation.

To compensate for an underlying encoding-consolidation defect w have found that, while depressed, patients overstructure (chunk events) so that they effectively reduce, well below channel capacity, the information load which requires processing. The behavioral cost of such overstructuring is to limit the amount of experience that can be stored and later recovered.

Drug and behavioral studies of memory consolidation. A number o studies have been initiated during the past year where drugs, such as a physostigmine and choline, have been used to help explore processes of memory consolidation. We have been able to establish that manipulation of the cholinergic system plays an important role in establishing memory traces. This has been demonstrated with cholinergic drug manipulation post-learning as well as treatments that precede information acquisition. Thi research has also been used as a contrasting system for explori memory-learning defects in the organic dementias as described below.

A number of studies have demonstrated that certain stimulus characteristics such as encodability, or semantic as opposed to acoustic processing, determine the delayed recall of information without necessarily affecting immediate recall characteristics. This research has also evolved as a set of findings which relates encodability of stimuli with the consist with which events can be retrieved from long-term memory store without altering random retrieval processes.

Post-stimulus arousal, as compared to pre-stimulus presentation arousal, facilitates the retrieval of the fine structure of higher order memory components stored in memory; just as in animal learning studies arousal appears to determine memory consolidation as a function of time x intensity of arousal treatment post-learning (Weingartner, Hall, Murphy, & Weinstein, 1976).

This facet of consolidation research has also been explored by manipulating sleep deprivation, REM sleep, and the time of learning in relation to the time when sleep occurs as these might be reflected in how much information might be acquired and later recalled. Sleep deprivation appears to induce SDL and also depresses how much information can be effectively stored in memory. When depressed patients are deprived of only REM sleep then this same pattern of deficits in memory consolidation is also evident. We are presently examining the effect of when learning occurs just before-or-after sleep, and where retrieval is attempted just before-or-after-sleep as a further strategy for exploring the role of sleep and sleep stages in altering brain state and, thereby, learning and recall processes.

Schizophrenic thought disorder. We have completed a series of studies in which we have attempted to define and contrast cognitive processes in young schizophrenics, patients who have been ill for a long time, and samples of schizophrenic subjects on and off antipsychotic medication as well as inappropriate control groups. We have found that the thought disorder that is evident in schizophrenia is determined by a defect in encoding processes which can be characterized as narrow-band information processing, an inability to appreciate and use context in organizing events, and a deficit in "chunking" processes that are necessary for reducing the total amount of information which must be processed (Weingartner, van Kammen, & Docherty, submitted for publication). We are presently using this research framework to review the numerous theories, models and empirical findings that have been accumulated to account for schizophrenic thinking. We have also begun to examine the effect of syntactic structure (in contrast to semantic organization) in determining schizophrenic thinking.

The structure of storage and retrieval of information in children. We have just begun to examine the amphetamine cognitive response in both normal children as well as in hyperactive children. Our preliminary findings appear to show that amphetamine does facilitate learning of verbal information but not pattern information and that this occurs in normal as well as in hyperactive children.

Learning and memory in the organic dementias. We have evolved a program that is designed to relate cognitive and biological factors through the study of patients with central nervous system disturbances as in the organic dementias. These have included studies of patients with Huntington's Disease (HD), Korsakoff's Psychosis, Alzheimer's Disease, and patients with other CNS lesions.

We have contrasted some of the cognitive effects of these different disturbances of brain function and attempted to acutely alter brain function in these patients with various drug manipulations (e.g., using scopolamine, physostigmine, and amphetamine).

We have found that HD patients 1) can not encode events so that consistent retrieval of events in memory is disrupted (Weingartner, Caine, & Ebert, in press). 2) The same events stored in memory appear to be recalled and forgotten at different points in time, (random retrieval) (Caine, Ebert, & Weingartner, in press). 3) These patients do not respond to cues, although their meaning may be appreciated, as a means of facilitating retrieval. 4) Disturbances in serial recall or retrieval of episodic events seem to be basic determinants of the thought disorder in the HD dementia (Weingartner, Caine, & Ebert, submitted for publication). 5) As yet, drugs tested have further disrupted cognitive performance in these patients and none has facilitated any of the components of information processing examined to date. We are still actively pursuing drug manipulations which might improve cognitive processe

Significance to Mental Health Research: The use of cognitive models has provided a powerful framework within which it is possible to describe important characteristics of schizophrenic disorders, depression, mania, organic dementias, and drug-altered states in patients and normal controls. This research effort has proved to be a particularly useful tool in relating biological and psychological phenomena as these are expressed in these different disorders. The clinical findings have also been useful in building more viable theories of how information is stored, learned, and retrieved and of the central nervous system determinants and representation of these behavioral events.

Proposed Course of Project: These research efforts are still in their infancy. We have uncovered much useful information about normal information processing, how it is altered in various clinical states, and the biological determinants that account for such cognitive processes and changes in those processes.

We propose to continue to pursue mechanisms focused on cognitive research by 1) emphasizing characteristics of processing strategi that are used to store information under various brain state conditions (we have, as yet, done little to directly characteriz such processes), 2) exploring drugs and other treatments that

might facilitate learning-memory in populations such as depressed patients, Huntington's Disease, Korsakoff's Psychosis, hyperactive children, and 3) examining in greater detail the biology of normal and disturbed information processing through manipulations that alter arousal, study of brain laterality (we are just now developing a collaborative proposal to accomplish this), and by exploring cognitive processes in patients with localized neuropathology.

Dissemination of Information:

Differential effects of marijuana and alcohol on hemispheric function. American Psychiatric Association, May, 1977.

Mood state dependent learning. American Psychiatric Association (VI World Congress), August, 1977.

Brain states and memory. American Psychological Association, September, 1976.

Storage and retrieval of experience while intoxicated. Conference on Alcohol and Memory (NIAAA Invited Address), September, 1976.

State dependent learning and drug retrieval. American Anthropological Society, November 1976.

Member Huntington's Disease Commission (U.S. Senate): Generate plans to research Huntington's Disease and apply findings to management, care and treatment of H. D.

Publications:

Stillman, R.C., Galanter, M., Fox, S., Lemberger, L., Weingartner, H. and Wyatt, R.J.: Tetrahydrocannabinol (THC): Metabolism and subjective effects. Life Sciences 19(4): 569-576, August 15, 1976.

Stillman, R.C., Eich, J.E., Weingartner, H. and Wyatt, R.J.: Marijuana induced state-dependent amnesia and its reversal by cueing. In Braude, M.C. and Szara, S. (Eds.): The Pharmacology of Marijuana. New York, Raven Press, 1976, pp. 453-456.

Weingartner, H., Hall, B., Murphy, D.L. and Weinstein, W.: Imagery, affective arousal and memory consolidation. Nature 263: 311-312, 1976.

Weingartner, H., Miller, H. and Murphy, D.L.: Mood state dependent retrieval of verbal association. J. Abnorm. Psychol. 86: 276-284, 1977.

Weingartner, H. and Murphy, D.L.: Brain states and memory: State dependent storage and retrieval of information. Psychopharmacol. Bull. 13: 66-67, 1977.

Caine, E.D., Ebert, M.H. and Weingartner, H.: An outline for the analysis of dementia: The memory disorder of Huntington's Disease. Arch. Neurol., in press.

Caine, E.D., Hunt, R.D., Weingartner, H. and Ebert, M.H.: Huntington's Dementia: A review of clinical and neuropsychological features. Arch. Gen. Psychiat., in press.

Weingartner, H.: Human state dependent learning. In Ho, Richards and Chute (Eds.): Drug Discrimination and State Dependent Learning. New York, Academic Press, in press.

Weingartner, W.: Storage and retrieval of experience while intoxicated. In Birnbaum, I. and Parker, E. (Eds.): Alcohol and Memory. New York, Academic Press, in press.

Weingartner, H., Caine, E.D. and Ebert, M.H.: Imagery, encodability and the retrieval of information from memory. J. Abnorm. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00496-01 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Animal Models of Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S.M. Anderson Staff Fellow LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous research has suggested a relationship between human psychopathology and levels of platelet MAO activity. Preliminary studies of variability in mouse MAO with age and sex are underway. The relationships between brain, liver and platelet MAO activity are also being investigated. Suggestions that MAO activity is related to general motor activity are being studied in genetically heterogeneous mice measuring activity in home cages with animex monitors. No research results are available at this writing.

Normal social interactions in small groups of mice have been and are being observed. We are attempting to develop new behavioral tests in a social situation for the assessment of drug response. We are particularly interested in developing an appropriate test for the effects of LSD, amphetamines, phenothiazines and MAO inhibitors on mouse behavior.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00497-01 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The development of an instrument that delineates the syndrome of Borderline Schizophrenia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Philippe Khouri, M.D. Research Psychiatrist

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1		

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eight items were empirically derived from an extensive review of the literature and a review of case histories from previous studies conducted by this laboratory on the subject of the schizophrenia spectrum. A questionnaire named "The Symptom Schedule for the Diagnosis of Borderline Schizophrenia" was applied to 31 cases histories (14 diagnosed borderline schizophrenia and 17 outside the spectrum). The Schedule reliably differentiated between the two groups.

PHS-6040

Borderline Schizophrenia is perhaps the most controversial diagnostic category in psychiatry. This study attempted to 1) validate the concept by demonstrating that it can be differentiated from neuroses and personality disorders on the one hand and chronic/true/process schizophrenia on the other hand, 2) determine the reliability of the individual items in the Schedule.

Three raters, who were blind to the hypothesis as well as to the individual diagnoses, scored 31 case histories on the 8 items. Each item can receive one of three scores: 0, 1 or 2. There were no false positive, i.e., none of the non-borderline schizophrenia cases had a score of 2 on any item. There was 1 case which was false negative. The reliability of the individual items was variable: 6 items were significant at a .01 level; 2 were not significan

This study is only a preliminary report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00498-01 LPP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Personality assessment of the first-degree relatives of schizophrenics.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Philippe Khouri, M.D.	Research Psychiatrist
OTHER:	Richard Haier, Ph.D.	Research Psychologist
	David Rosenthal, Ph.D.	Research Psychologist

COOPERATING UNITS (if any)

Adult Psychiatry Branch

LAB/BRANCH

Laboratory of Psychology & Psychopathology

SECTION

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1		

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

First-degree relatives of schizophrenics have been described in the clinical literature as having certain personality abnormalities like "introversion, suspiciousness, lacking in interpersonal effectiveness..." This study selects 60 first-degree relatives of schizophrenics and a control group of 60 first-degree relatives of manic-depressives. The Research Diagnostic Criteria (RDC) system is used to select the two groups. Three personality tests, the California Psychological Inventory (CPI), the Myers-Briggs and the Cattell 16PF are used to assess the personality characteristics that may be specific to individuals biologically related to a group of schizophrenics.

PHS-6040

Project Description

Ever since E. Kraepelin and E. Bleuler reported that some relatives of schizophrenics have certain kinds of peculiarities in their personalities variously described as "eccentric, irritable, introvert, lacking in interpersonal effectiveness, suspicious, odd ...," many reports about the psychological characteristics of relatives of schizophrenics have appeared in the literature. Some reports are anecdotal, like those of Kraepelin and Bleuler, while others used projective tests like the Rorschach or objective type of tests like the MMPI.

Singer and Wynne were able to discriminate the parents of schizophrenics from those of neurotic and normal subjects, using the Rorschach. Other investigators, however, were unable to confirm their findings.

A study by Cohler, et al., on the parents of schizophrenics, neurotics, "borderline personalities" and normals revealed no difference in the MMPI profiles of the four groups.

There are two possible explanations for these negative results:

1. The number of relatives of each group was too small to show any significant difference between the MMPI group profiles.
2. The MMPI is standardized against clinically sick and hospitalized populations and may not be suitable for use in a population where most members are not hospitalized for gross psychopathology as in the relatives mental patients in general.

In the proposed study we will use objective personality tests that are standardized against non-clinical populations but measure personality characteristics like introversion/extraversion, interpersonal effectiveness, suspiciousness/trust, sociability, etc.

The three tests we will use are: The California Psychological Inventory (empirically derived), The Myer-Briggs (rationally derived) and the Catell 16PF (factor-analytically derived).

If significant differences are found among the first-degree relatives of both groups, a sample of subjects from both groups with controls from within each group will be recalled and given a semi-structured psychiatric interview with the CAPPS. This would allow us to correlate certain personality traits with clinical psychopathology.

Twenty patients from the Clinical Center and St. Elizabeth's Hospital, with the diagnosis of schizophrenia, and 20 patients with the diagnosis of manic disorder will be selected. The probands selected will either be hospitalized or former patients of the wards. However, their inclusion in the study will be done in coordination with the clinical staff or the therapists seeing them. Each patient's diagnosis will be checked against the Research Diagnostic Criteria of Spitzer, Endicott, and Robins, and each patient

should have first-degree relatives in the Washington area. With the knowledge of the patient, all his/her first-degree relatives (over the age of 15) in the Washington area will be contacted (first by mail, then by phone) and asked to come to the National Institutes of Health in Bethesda, for a three-hour session. During this session the three personality tests will be administered. The tests may be given individually or in groups; all relatives completing the session will receive standard payment. We expect to test an average of 3 relatives per patient. The patient also will be asked to complete the same tests under the supervision of ward personnel (the items on all three tests are very similar to MMPI items, and should present no particular problem.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00671-08 LSES

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Social Origins of Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard I. Pearlin	Research Sociologist	LSES NIMH
Other:	Carmi Schooler	Research Social Psychologist	LSES NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.10	1.10	1

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research examines the relationship between social experience and psychological well-being. It looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two points in time. In 1972 a sample of 2300 people, representative of the Chicago Urbanized Area were interviewed and a sub-sample (1200) of this group was reinterviewed in 1976-77. One analysis of these data concerns the conditions underlying the greater tendency toward depression among women than among men; a second inquiry is directed at the circumstances differentially disposing people of different marital status toward depression; a third deals with some of the circumstances leading to stress in marriage; another is concerned with the use of alcohol as a mechanism to manage anxieties arising out of economic problems; and a final analysis aims at identifying patterns of coping employed by people in contending with different kinds of life strains.

Project Description:

This is a longitudinal study of social stress whose data were collected at two points in time spanning a period of about four years. In 1972 interviews were conducted with a representative cluster sample of 2300 adults in the urbanized area of Chicago. These interviews sought information about three features of people's lives: 1)the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; 2)the coping strategies people employ in dealing with life-problems; and 3)symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 people drawn from those originally interviewed. Many of the questions initially asked about the chronic role problems, coping, and psychological distress and these were repeated, thus providing longitudinal information about stability and change on these dimensions of people's lives. The second interview, in addition, sought information about potentially distressful types of life-strains that were not included in the 1972 interviews. One type is represented in the many events and role transitions that are normative aspects of life-cycle--e.g., being married, having a child, retiring from work, etc. A second type of strain is also a concomitant of commonly experienced events, but these events are less expected and more eruptive, such as divorce, illness, involuntary unemployment, and so on.

Together the two interviews provide a large body of information about problems woven into the fabric of major social roles, the gains and losses involved in life-cycle transitions, the crises that can intrude into people's lives, coping repertoires and psychological outcomes. It is the long-range goal of the research to reconstruct from this information those elements of social experience that are most deleterious to mental health and to learn how people attempt to avoid being psychologically harmed by such experience.

The work of the past year proceeded along two lines: the continuation of the analysis of the 1972 data and the collection and preliminary examination of the 1976-77 data. Analysis of the earlier material largely centered on identifying factors that raise or lower self-esteem. The understanding of self-esteem is important within a larger study of social stress, for earlier investigation shows positive self-regard to be a barrier to the stressful consequences of life-strains. Central to the analysis is the influence of social stratification on self-esteem. It was learned that the level of adults' self-esteem is associated with the level of their education, their occupational status and their income. Much of the previous research looking for these kinds of relationships were aimed at school-age children, and it has consistently failed to find any connection between stratification and self-esteem. For this reason, the inquiry sought to identify why adult self-esteem is influenced by social and economic inequality, but not that of children. One reason is that for children location in broadly stratified systems has no reference to personal success or failure. In contrast, adults who are competing in society's opportunity structures are likely to see their location as a direct reflection of their abilities and, depending on where they are, will either suffer decrements or enjoy increments to self-esteem. This is

especially the case for adults who value achievement and status. Another factor differentiating the relevance of stratification to the self-esteem of children and adults concerns the nature of adults' occupational experience. Being under the direction of others and having little opportunity for self-direction are known from other research to be inimical to self-esteem. These are conditions most often experienced by blue-collar workers, thus helping to account for observed differences in self-esteem among people of different occupational status. Children, of course, are not yet exposed to these conditions, with the result that their parents' occupational status has little bearing on their self-esteem. Finally, the social world of children, centered as it is around community and school, has much less status differentiation than that of adults. Consequently, children cannot make the same kinds of comparisons between themselves and others as adults who are more likely to be in direct or indirect contact with people of different status. Status differences, being part of the awareness of adults, are therefore more apt to be the basis of invidious comparisons and a source of their self-esteem. In these ways, social stratification comes to affect the development of a crucially important personality characteristic.

The 1976-77 follow-up interviews with the 1972 sample are now completed. Although there was but approximately a four-year interval between interviews, almost a third of the respondents had moved from their original addresses, about half of these leaving the state. Young, minority subjects were found to be particularly mobile. This placed an added burden on the organization of the field work and on devising procedures for tracing people. But although the follow-up required more time and effort than originally anticipated, interviews were successfully completed with an acceptable sub-sample.

The first analysis of the follow-up data, presently in its preliminary stages, is concerned with the relative impact on psychological well-being of the three types of life-strains for which we now have information: the chronic role problems, role transitions and eruptive crises. Findings are emerging that should result in some modification of prevailing thinking. One such finding is that the normative transitions that occur in occupation and family roles are the least provocative of emotional distress. This is contrary to a widely held view that the major stresses of adult development and of aging result from the gain and loss of roles. Instead, it is the durable problems of daily life and the eruptive crises with which people have to contend that are most likely to create distress. A second finding goes against the grain of still another commonly held assumption; namely, that any life-change, regardless of its nature, results in stress because change always demands some degree of readjustment. This does not appear to be the case, for voluntary changes--such as leaving the labor force to have children, moving to another job, or promotion are unrelated to emotional distress. Indeed remarriage by formerly divorced or widowed people is a change that more than any other is associated with psychological well-being. Thus, as we begin to specify the nature and structure of people's experience, we can better understand social influence on mental health.

In the coming year this inquiry will continue to reveal in detail both the psychological consequences of different life-strains in major social roles and the conditions that mediate these consequences. We shall also be dealing with changes captured by our longitudinal data, attempting to establish relationships between external social circumstances and inner emotional life both through space and time.

Publications:

Pearlin, Leonard I.: Sex Roles and Depression. In: Datan, N. and L. Ginsbert, (Eds.): Life-Span Developmental Psychology Conference: Normative Life Crises, 1974. New York, Academic Press, 1975, pp. 191-207.

Pearlin, Leonard I.: Status Inequality and Stress in Marriage. American Sociological Review, 40 (June), 1975:344-357.

Pearlin, Leonard I. and Clarice Radabaugh: Economic Strains and the Coping Functions of Alcohol. American Journal of Sociology, 82 (Nov.), 1976:652-663.

Pearlin, Leonard I. and Joyce S. Johnson: Marital Status, Life-Strains and Depression. American Sociological Review. In press.

Pearlin, Leonard I. and Carmi Schooler: The Structure of Coping. Journal of Health and Social Behavior. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00672-12 LSES

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Social Psychological Correlates of Occupational Position

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. L. Kohn	Chief, Lab. of Socio-environmental Studies	LSES NIMH
OTHER:	C. Schooler	Research Psychologist	LSES NIMH
	J. Miller	Staff Fellow	LSES NIMH
	K. Miller	Staff Fellow	LSES NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.20	3.70	6.50

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the re-interviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children.

PHS-6040
(Rev. 10-76)

The principal goal of this research is to assess the effects of occupational conditions on psychological functioning. The research began with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to our specifications by the National Opinion Research Center of the University of Chicago in the spring and summer of 1964. In 1974, the National Opinion Research Center conducted follow-up interviews, again to our specifications, with a randomly-selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a randomly-selected child.

One essential purpose of the follow-up study is to provide more definitive data on causal processes than could be provided by a single cross-sectional survey. With these data, we hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of the wives is designed to ascertain whether occupational conditions affect men and women similarly. These data should also enable us to assess the effects of men's occupational experiences on their wives' psychological functioning and of women's occupational experiences on their husbands' psychological functioning, in each case taking account of the individual's own occupational experiences. The study of the children is designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, our major research efforts have been focused on four objectives: (1) Analyzing the reciprocal relationship between the substantive complexity of men's work and their intellectual flexibility; (2) Developing measurement models for other dimensions of occupational structure and other facets of psychological functioning; (3) Assessing the effects of occupational conditions on the psychological functioning of women; and (4) Preliminary analyses of the determinants of social mobility.

1. Analyzing the reciprocal relationship between the substantive complexity of men's work and their intellectual flexibility. Our earlier work, based on cross-sectional analysis, employed an econometric technique called two-stage least-squares to assess the reciprocal effects of job conditions and psychological functioning. In particular, we assessed the magnitude of the reciprocal effects of the substantive complexity of work and many facets of psychological functioning: occupational commitment, job satisfaction, parental valuation of self-direction, anxiety, self- esteem, receptiveness or resistance to change, standards of morality, authoritarian conservatism, intellectual flexibility, the intellectual demandingness of leisure-time activities,

and three types of alienation--powerlessness, self-estrangement, and normlessness. The substantive complexity of work affects each of these facets of psychological functioning, in most cases more than that facet of psychological functioning affects the substantive complexity of work. This evidence is not definitive--only longitudinal studies of real change in real people can provide definitive evidence--but it does establish a strong prima facie case that the substantive complexity of work has a real and meaningful effect on a very wide range of psychological phenomena.

More definitive (albeit less extensive) evidence comes from the first major analysis of the data of the follow-up study we conducted with a representative sub-sample of the men in the original study. This analysis focuses on the reciprocal relationship between one pivotal dimension of occupational structure, the substantive complexity of work, and one pivotal dimension of psychological functioning, intellectual flexibility. We choose the substantive complexity of work as the occupational condition to be assessed in this analysis for three reasons: We have found substantive complexity to be a central element of occupational structure--an important determinant of occupational self-direction and an important "structural imperative" of the job. Moreover, substantive complexity is as strongly correlated with psychological functioning as is any other dimension of occupation we have examined. Finally, we have excellent descriptive information about the substantive complexity of work, which we have been able to validate by comparison to the objective job assessments of trained occupational analysts.

We choose intellectual flexibility as the aspect of psychological functioning to be assessed in this analysis in part because it offers us the greatest challenge--intellectual flexibility obviously affects recruitment into substantively complex jobs, and there is every reason to expect it to be one of the most resistant to change of all facets of psychological functioning we have measured. Moreover, intellectual flexibility--though not much studied by sociologists--is so important a part of psychological functioning that we must not unthinkingly assume it to be entirely the product of genetics and early life experience. Rather, we should empirically test the possibility that intellectual flexibility may be responsive to the experiences of adult life.

Analyses of longitudinal data are immensely difficult, because they require the development of "measurement models" that separate unreliability of measurement from real change in the phenomena studied. For example, we need to be able to assess how much of the apparent change in the substantive complexity of the men's work represents real change in men's job conditions over the 10-year interval between the initial survey and the follow-up survey, and how much is a function of unreliability in the measuring instruments. Similarly, we need to be able to assess how much of the apparent change in intellectual flexibility represents real change in the men's cognitive functioning

and how much is a result of measurement error.

Recently, Jöreskog and his associates have introduced powerful new procedures for using maximum likelihood confirmatory factor analysis to separate unreliability from change in causal analysis. The essence of these measurement models is the use of multiple indicators of each of the principal concepts. Using confirmatory factor analysis, it is possible to infer from the covariation of the indicators the degree to which each reflects the underlying concept that they all are hypothesized to reflect, and the degree to which each reflects anything else, which for measurement purposes is considered to be error. The test of our success in differentiating "true scores" on the underlying concept from error in the indicators is how well the measurement model reproduces the original variance-covariance matrix.

The first step in the use of these procedures is to develop "measurement models" for the principal concepts; these models will later form the basis for the causal analysis. The measurement models must allow for unreliability in all the items that measure a concept, and also allow for these errors being correlated in repeated measurements of the same phenomena. For example, any errors in the information obtained in 1964 on, say, the complexity of men's work with data, or in our coding of that information, might well be correlated with errors in the same type of information in 1974. Such correlated error in any constituent item might make our index of substantive complexity seem more stable or less stable than it really is. Before assessing changes in the levels of substantive complexity of men's work, and the reasons for these changes, we must remove the effects of correlated errors in measurement.

We have constructed such measurement models for both the substantive complexity of work and intellectual flexibility. The models show the over-time stability of both variables to be high, that of intellectual flexibility especially so: the correlation between men's intellectual flexibility at the time of the original study and their intellectual flexibility ten years later, shorn of measurement error, is 0.93. Nevertheless, the reciprocal effects of substantive complexity and intellectual flexibility are considerable.

The effect of substantive complexity on intellectual flexibility is approximately one-fourth as great as the effect of men's 10-year earlier levels of intellectual flexibility. This effect is essentially contemporaneous: the lagged path from 1964 substantive complexity to 1974 intellectual flexibility is a statistically nonsignificant 0.05, while the contemporaneous path from 1974 substantive complexity is a more substantial and statistically significant 0.18.

A path of 0.18 might not in ordinary circumstances be considered especially striking; but a continuing effect of this magnitude on so stable a phenomenon as intellectual flexibility is impressive, for the

cumulative impact is much greater than the immediate effect at any one time. Continuing effects, even small-to-moderate continuing effects, on highly stable phenomena become magnified in importance. The effect of the substantive complexity of work on intellectual flexibility is especially noteworthy when we take into account that we are dealing with men no younger than 26 years of age, who are at least ten years into their occupational careers.

The reciprocal effect of intellectual flexibility on substantive complexity is even more impressive--surpassing that of the substantive complexity of the 1964 job. This effect is entirely lagged, i.e., it is the men's intellectual flexibility in 1964, not contemporaneously, that significantly affects the substantive complexity of their 1974 jobs. The longitudinal analysis thus demonstrates something that no cross-sectional analysis could show -- that, over time, the relationship between substantive complexity and intellectual flexibility is truly reciprocal. Substantive complexity has a more immediate effect on intellectual flexibility: today's job demands affect today's thinking processes. Intellectual flexibility, by contrast, has a delayed effect on substantive complexity: today's intellectual flexibility has scant effect on today's job demands, but it will have a sizeable effect on the further course of one's career. The cross-sectional analysis portrayed only part of this process, making it seem as if the relationship between the substantive complexity of work and psychological functioning is mainly unidirectional, with work affecting psychological functioning but not the reverse. The longitudinal analysis portrays a more intricate and more interesting, truly reciprocal, process.

The data thus demonstrate, beyond reasonable doubt, what heretofore could be stated as only a plausible thesis buttressed by presumptive evidence--that the substantive complexity of men's work both considerably affects, and is considerably affected by, their intellectual flexibility.

These findings come down solidly in support of those who see occupational conditions as affecting personality and in opposition to those who see the relationship between occupational conditions and personality as resulting solely from selective recruitment and job-molding. We do not deny that personality has great importance in determining who go into what types of jobs and how they perform those jobs; in fact, our analyses underline the importance of these processes. But that has never been seriously at issue. What has been disputed is whether the reverse phenomenon--of job conditions molding personality--also occurs. The evidence of our study unequivocally supports the position that it does occur. Thus our findings bear directly on an issue central to the field of social structure and personality-- whether social-structural conditions affect personality only through childhood socialization or continue to affect personality throughout the life span. Here is clear evidence that one important facet of social structure-- the substantive complexity of work--directly

affects adult personality.

In particular, this study adds to and helps specify the growing evidence that the structure of the environment has an important effect on cognitive development and that cognitive processes do not become impervious to environmental influence after adolescence or early adulthood, but continue to show "plasticity" throughout the life span. Our findings reinforce this conclusion by showing that intellectual flexibility continues to be responsive to experience well into mid-career and probably beyond. In fact, it appears that the remarkable stability of intellectual flexibility reflects, at least in part, stability in people's life circumstances. Intellectual flexibility is ever-responsive to changes in the substantive complexity of people's work; for most people, though, the substantive complexity of work does not fluctuate markedly.

This study demonstrates as well the importance of the impact of intellectual flexibility on substantive complexity. We think it noteworthy that this effect appears to be lagged, rather than contemporaneous. The implication is that the structure of most jobs does not permit any considerable variation in the substantive complexity of the work: job conditions are not readily modified to suit the needs or capacities of the individual worker. Over a long enough time, though--certainly over a period as long as ten years--many men either modify their jobs or move on to other jobs more consonant with their intellectual functioning. Thus, the long-term effects of intellectual flexibility on substantive complexity are considerable, even though the contemporaneous effects appear to be negligible.

In the broadest sense, our findings support our general strategy for studying the relationship between social structure and personality. We have consistently argued that, in interpreting the relationship between social structure and individual psychological functioning, one should always ask how a person's position in the larger social structure affects the conditions of life that directly impinge on him. Thus, in attempting to interpret the relationship between social class and values and orientation, we saw class-correlated differences in occupational conditions as a potentially important bridge between position in the hierarchical ordering of society and conceptions of reality. Substantive complexity is particularly important, for on the one hand, the substantive complexity of work is closely linked to the job's location in the stratificational system; and on the other hand, the substantive complexity of people's work is correlated with their values and orientation. Our past research demonstrated that the relationship between social class and values and orientation could reasonably be attributed, in large degree, to class-correlated differences in such occupational conditions as substantive complexity. But not even our two-stage least squares analysis demonstrated conclusively that substantive complexity has an actual causal effect on values, orientation, or any other psychological phenomenon. The present

analysis buttresses our analytic strategy by showing that substantive complexity actually does have a causal impact on one pivotal aspect of psychological functioning, intellectual flexibility.

Admittedly, our research has not yet demonstrated that substantive complexity directly affects values or self-conception or social orientation-- in fact, anything other than intellectual flexibility. Still, intellectual flexibility is the crucial test. Because of its remarkable stability, intellectual flexibility offers the most difficult challenge to the hypothesis that substantive complexity actually affects some important aspect of psychological functioning. Moreover, intellectual flexibility is tremendously important in its own right. Finally, we see intellectual flexibility as intimately related to values, self-conception, and social orientation. It is, in fact, an important link between social class and self-directed values and orientation. Thus, demonstrating the causal impact of substantive complexity on intellectual flexibility gives us every reason to expect substantive complexity to have a causal impact on values and orientation, too. In our further analyses, we shall assess the hypothesized causal impact of substantive complexity--and of other structural imperatives of the job-- on values, self-conception, and social orientation. For now, one crucial causal link in the relationship between social structure and psychological functioning has been conclusively demonstrated.

2. Developing measurement models for other dimensions of occupational structure and other facets of psychological functioning. The next step in this analysis is to extend the causal model from one occupational condition, the substantive complexity of work, and one facet of psychological functioning, intellectual flexibility, to a much more general model of the interrelationship of occupational structure and psychological functioning. To this end, we have constructed measurement models for such dimensions of occupational structure as closeness of supervision and routinization of work, and for such facets of psychological functioning as self-esteem, anxiety, authoritarian conservatism, receptiveness or resistance to change, and standards of morality. We are now in process of combining these various measurement models, so that we can estimate the correlations among the various concepts, shorn of measurement error. This step in the research process is both tedious and demanding; but it is essential. Once past this, we shall be in position to get underway in the intended large-scale causal analysis of the reciprocal effects of occupational structure and psychological functioning.

3. Assessing the effects of occupational conditions on the psychological functioning of women. Formal employment is clearly becoming a more common life experience for women. The rate of female labor force participation has been steadily increasing, particularly among married women with children. In 1970, over 40% of the adult female population was employed.

The 1974 follow-up survey of men in the civilian labor force included an interview with the wife of those men who were then married. Measures of psychological functioning and job histories were obtained in a form closely parallel to that of the men's interviews. The sample is not altogether representative of all married women in the United States because all these women were married to men between the ages of 25 and 65. Nonetheless, useful estimates of the relationship between work and the psychological functioning of women will be obtained if this sampling bias is kept in mind. These women's status as wives of a national sample of men is useful for placing psychological development in the context of family life. Not only will the impact of work on the individual be assessed, but the interrelationship of husband and wife can be examined. A major question at hand is whether (and under what circumstances) men's occupational experiences affect their wives' psychological functioning and whether (and under what circumstances) women's occupational experiences affect their husbands' psychological functioning. The collection of data on wives is also useful for investigating the transmission of values and social orientation to children, for such an analysis requires information on the background, job conditions, and psychological development of both parents.

The first phase of the analysis of the women's data has been to determine whether the questions used to measure intellectual flexibility, self-conception, values, and social orientation had the same meaning for women as for men. For example, the questions that were used to index father's values for their children have been shown to distinguish fathers who stress self-direction from those who place more importance on conformity to external authority. In order to compare the effect of occupational conditions on men's and women's parental values, the same questions must index the same dimensions of valuation for mothers. It is possible, for example, that mothers' values might center on interpersonal relationships or aspirations instead of on self-direction and conformity to external authority. If the same questions tap an entirely different concept for women, statistical comparisons become much more difficult.

Our analysis shows that all the major measures of values, self-conception, social orientation, and intellectual functioning have the same factor structure for women as for men. This finding not only simplifies the research strategy of the study, but further verifies the universality of the concepts.

The next step in the analysis is to assess the impact of job conditions of formally employed women on psychological functioning. The strategy for this inquiry, now in progress, is to begin by establishing the overall relationship between work and psychological development for women, and then to investigate special circumstances that may alter this relationship. Two important considerations for working women are full- versus part-time employment and the level of occupational commitment.

One might expect the effect of occupational conditions to be muted if the work is not full-time or not of central importance to the individual.

4. Preliminary analysis of the determinants of social mobility. The data of this study are also being used to study social mobility. Although a great deal of sophisticated research has previously been carried out in this area, the size of our sample and the depth and extensiveness of our data permit us to replicate and substantially extend the work of others. Thus, the size of the 1964 sample permits us to do sub-population analyses. We can compare the mobility processes of old and young, black and white, and urban and rural populations. We are also able to considerably enlarge the list of social characteristics that may serve as predictors of attained occupational status. We have done this by including in our mobility models maternal and paternal grandfathers' occupational status, mother's occupational status, and such characteristics of the individual's childhood as the religion and region of the country in which he was reared.

We are also in a position to take an unusually intensive look at inter-generational mobility, because we have both extensive background information and also unusually detailed job histories. Since we have such job histories, we can analyze not only the determinants of the job the individual held at the time of the follow-up study in 1974, but also determinants of the job he held at the time of the initial interview in 1964, and of the first job he held for 6 months or longer. Furthermore, since we have extensive data on many aspects of the individual's value system and psychological functioning in 1964, we can see how such characteristics affect his occupational mobility to 1974.

All of these analyses have been begun, but have not yet been carried far enough to present conclusions. Analyses have also been performed on the psychological effects of mobility. Here, our results, though incomplete, seem fairly firm. We have not been able to locate any psychological effects of mobility that are independent of the effects of the individual's social origins and his present social status. Mobility, per se, seems to have little or no psychological impact.

Publications:

Kohn, M.L.: Occupational Structure and Alienation. American Journal of Sociology, 82:111-30, 1976.

Kohn, M.L.: Social Class and Parental Values: Another Confirmation of the Relationship. American Sociological Review, 41:538-45, 1976.

Kohn, M.L.: Looking Back--a 25 Year Review and Appraisal of Social Problems Research. Social Problems, 24:94-112, 1976.

Kohn, M.L.: Reassessment, 1977. In M. L. Kohn, Class and Conformity: A Study in Values. Second Edition. Chicago: University of Chicago Press, 1977, in press.

Kohn, M.L. and Schooler, C.: The Reciprocal Effects of the Substantive Complexity of Work and Intellectual Flexibility: A Longitudinal Assessment. American Journal of Sociology, in press.

Suzman, R., Miller, K. A., and Charrad, M.: Employment Effects on Fertility Control in Tunisia. Pp. 91-113 In Rosenberg, S. (Ed.), Recent Empirical Findings on Fertility: Korea, Nigeria, Tunisia, Venezuela, Philippines. Washington, D. C., Interdisciplinary Communications Program, Smithsonian Institution, 1976.

Schooler, C.: Serfdom's Legacy: An Ethnic Continuum. American Journal of Sociology, 81:1265-86, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00673-14 LSES

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Family Role Values and Behavior in Japan

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Schooler	Research Psychologist	LSES NIMH
OTHER: K. C. Smith	Sociologist	Univ. of Pittsburgh

COOPERATING UNITS (if any)
Mental Health Center, Kobe, Japan

LAB/BRANCH
Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.10	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This year saw the final preparation for publication of papers dealing with the effect of social structure and culture on Japanese women's role values and performance.

PHS-6040
(Rev. 10-76)

Project No. Z01 MH 00573-14 LSES

This past year saw the preparation and submission for publication of two papers reporting on how social structure and culture affect Japanese women's role values and their behavior as mothers and wives. The contents of these papers were reported on extensively in last year's annual report. Present plans call for the termination of this study when both papers are accepted for publication.

Publications:

C. Schooler and K. C. Smith
" ... and a Japanese wife": Social structural antecedents of women's role values in Japan. SEX ROLES: A Journal of Research. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00674-07 LSES

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Social, Psychological, and Biological Interactions in Schizophrenics and Normals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Schooler	Research Psychologist	LSES	NIMH
OTHER: T. Zahn	Research Psychologist	LP	NIMH
M. S. Buchsbaum	Research Psychiatrist	APB	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology, NIMH
Adult Psychiatry Branch, NIMH

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This year has seen the extension of this line of investigation through the processing of experimental data aimed at clarifying the relationship between amine oxidase activity and psychological functioning, as well as the examination of the psychological correlates of dopamine-beta-hydroxylase. Present analyses have not progressed far enough to permit the reporting of conclusions.

PHS-6040
(Rev. 10-76)

For the past several years, this study has investigated the relationship between blood chemistry and individual functioning. Last year, this study presented findings on the relationships between amine oxidase activity and psychological functioning in normals which were seen to have implications about the role of amine oxidase activity in schizophrenia and manic-depressive behavior.

This year has seen the extension of this line of investigation through the processing of experimental data aimed at clarifying the relationship between amine oxidase activity and psychological functioning, as well as the examination of the psychological correlates of dopamine-beta-hydroxylase. Present analyses have not progressed far enough to permit the reporting of conclusions.

Publications:

Schooler, C., Buchsbaum, M. S., and W. T. Carpenter, Jr. Evoked response and kinesthetic measures of augmenting/reducing in schizophrenics: Replications and extensions. Journal of Nervous and Mental Disease. 163(4): 221-232, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00677-15 LSES

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

HEALTH ORIENTATIONS OF PARENTS AND CHILDREN

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: John D. Campbell, Ph. D., LSES/NIMH
Research Psychologist

Other: Caren M. Carney, LSES/NIMH
Psychologist

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this research on health orientations of parents and children are first, to describe variations in attitudes, values, beliefs, perceptions, and behavior concerning phenomena of illness and health; and second, to examine how selected personal and situational factors relate to these orientations. Individual perspectives on health, illness and illness-related stress, and the social role of the sick person are to a considerable part learned, and much of this social learning takes place in the interpersonal relationships of family life. Systematic research can shed light on this aspect of socialization, the process by which health orientations are acquired and modified.

PHS-6040
(Rev. 10-76)

This research examines children's and mothers' perspectives on illness and health. Information was obtained from 264 hospitalized children and their mothers by use of focused interviews, questionnaires, and rating scales. Interviews with children and mothers, and questionnaires completed by mothers provided data on (a) aspects of their definitions of illness, (b) reports of their own and other family members' feelings and actions when ill, (c) their attitudes and beliefs about illness prevention and treatment, (d) their values in selected areas not directly relevant to health, and (e) their views about appropriate standards of behavior (i.e., what should or should not be done in circumstances pertaining to illness and health).

A major goal of this research is to clarify the manner in which health orientations are acquired. It is a premise of this study that the largely stable variations in adults' views of health and illness are to an appreciable extent the products of a social process that begins in childhood. Influences contributing to these perspectives extend well beyond those of specific health-relevant experiences; the factors at work are integral to children's socialization more generally viewed. The process leading to individual variations in health orientations is in considerable part determined by social characteristics of the child and family members, systematic differences in conditions of life, and associated differences in general parental values, role expectations, and situation-specific norms.

Analyses completed this past year have helped to clarify the process leading to individual differences in the extent to which children expose themselves to risks that might place their health at hazard, a matter of obvious importance to personal well-being. Two different types of risk taking reported by children have been considered: (a) taking chances in active play that might lead to physical injury, and (b) risking illness by failing to follow simple hygienic or health maintenance routines. These are logically and empirically related types of risks, and they share some antecedents. But factors strongly influencing one aspect of risk do not necessarily influence the other. Consideration of similarities and differences in effects of possible antecedents provides useful information concerning the acquisition of orientations to health-relevant risks.

For both areas of risk taking, older children are more willing than younger ones to chance minor hazards to health and safety; this is in line with developmental expectations. General stereotypes concerning sex-role development would also suggest boys, not girls, as risk takers. This proves to be clearly the case for risks of injury, but whether the child is a boy or girl is almost irrelevant to whether simple hygienic conventions are neglected. Parental socioeconomic status, with its implications of possible differential access to health-care resources in case of harmful consequences of risk, did significantly influence both risks of injury and health maintenance risks--but the latter considerably more than the former. The possibility of parental observation and supervision is much greater for health maintenance than for playtime activity; for the latter, the child's peers may be the ones who exert pressure to bring risk performance in line with age- and

sex-role expectations. Supporting this is evidence that, though sibling structure has almost no utility in predicting whether the child neglects health-maintenance routines, children with many siblings (especially older siblings) are much more likely to take risks at play than are children lacking many brothers or sisters. Serving both as role models and enforcers, older siblings and perhaps other children as well command the playing field. Parents, on the other hand, hold sway at home.

In the growing child's expected progress toward adult roles, parents obviously play a critical part in the process. In risk taking, as in other areas, it is likely that they serve as role models and that by sanctions and encouragement they directly reinforce action. But such contributions to health-relevant risk taking of their children also reflect two different, largely independent aspects of parents' psychosocial orientations: (a) general social values that the parent holds for the child, and (b) much more specific, situation-relevant standards for the child's functioning in circumstances that might place health at risk. Children's reports of taking chances with illness or injury do show the influence of both of these aspects of parental orientations, but there are differences in the chain of effects. Mothers' standards for risks in health maintenance areas reflect family position in the socioeconomic structure, perhaps implying consideration of potential differential economic consequences of illness. And these situation-specific norms of the mothers significantly influence the child's own standards and, as a consequence, affect whether the child does or does not follow health-maintenance routines. For the other area of risk, chancing injury while at play, these specific maternal standards exercise little influence. Despite their being unrelated to standards concerned with health maintenance, parents' general values for their children (that is, broad goals that clearly reflect parental characteristics and expectations linked to the child's functioning in age and sex roles) also have a considerable influence on risk-taking behavior reported by children. As mentioned in a previous annual report, influence of these maternal values is contingent on the child's age; the impact of general parental goals increases markedly as children grow older; parental values affect both areas of health-relevant risk in this manner.

These contrasts between effects of mothers' specific health-relevant standards and their more general social values suggest that in the transmission of orientations from parent to child two different factors may be at work, one to a considerable degree situation-specific, the other much more dependent on the child's cognitive interpretations: (a) Parental control over particular situations, and the consequent possibility for direct parental reinforcement would contribute to children's acquisition of maternal normative standards pertaining to specific health risks. But one would not expect these standards to be generalized to other risks unless the child detected underlying situational similarities. (b) The child's acceptance of parental values has the potential for much more general application to diverse areas of action. But this acceptance may require more active participation by the child in the socialization process, and it may proceed at a different rate

from the acquisition of situation-specific norms, a rate dependent on cognitively mediated factors. The child, responding perhaps to subtler clues of parental perspectives, must construct generalized rules largely consonant with parental values and applicable to a wide range of social behavior including those that may involve risk taking; this process of induction shows orderly age-related changes that parallel cognitive growth.

Publications:

Campbell, J. D. Illness is a point of view. Nursing Digest. 4(3): 56-59, 1976.

Campbell, J. D. The child in the sick role: Contributions of age, sex, parental status, and parental values. Journal of Health and Social Behavior. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00851-13 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Brain Mechanisms of Species-typical, Prosematic Behavior in Squirrel Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T. K. Bussard	Biologist	LBEB NIMH
	R. E. Gelhard	Biologist	LBEB NIMH
	L. Waters	Biological Lab. Technician	LBEB NIMH

COOPERATING UNITS (if any)

John D. Newman, NICHD, Behavioral Biology Branch

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	.4	1.6

CHECK APPROPRIATE BOX(ES)

[] (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

[] (a1) MINORS [] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striatal complex constitutes the foundation of the forebrain of mammals and is believed to represent an evolutionary inheritance from mammal-like reptiles. The present project is part of a continuing study that is testing the hypothesis that the striatal complex plays a basic role in the organized expression of species-typical behavior. In the current experiments focusing on tegmental structures and pathways associated with the striatal complex, it has been found that bilateral lesions of the central tegmental tract in the midbrain leads to a fragmentation and eventual elimination of the species-typical mirror display of squirrel monkeys. In some cases, tegmental lesions near the diencephalic junction have been complicated by altered vocalization. In one animal, for example, the sound spectrogram showed that the isolation peeps had an infantile pattern.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The striatal complex constitutes a large part of the forebrain of mammals and is believed to represent an inheritance from long-extinct mammal-like reptiles. A primary purpose of this Laboratory is to test the hypothesis that the striatal complex plays a basic role in organizing the expression of species-typical, prosematic behavior. In preceding experiments it was shown that lesions of the medial pallidal segment of the striatal complex or its projecting pathways resulted in an enduring elimination or fragmentation of the species-typical, mirror-display behavior of gothic-type squirrel monkeys. The present experiments are part of a continuing study on the effects of destroying structures of the tegmentum that receive projections from the globus pallidus.

Methods Employed: The mirror display test for male gothic-type squirrel monkeys was described in previous project reports (see M-NP-LI-17, 1964). Of the five features of the display, vocalization, thigh-spreading, and forward thrusting of the erect phallus are the major and most regularly occurring manifestations, constituting what is referred to as the trump display. After animals reach criterion in 30 or more trials, symmetrical electrocoagulations are placed in the thalamic or midbrain tegmentum.

Major Findings: In further experiments on the diencephalic tegmentum an attempt was made to produce lesions involving midline areas in which stimulation is known to result in vocalization. In addition to leading to an elimination of display behavior such lesions resulted in abnormal vocalizations. Spectrographic analysis revealed changes ranging from the production of infantile sounds (otherwise found only in the first natal year) to an inability to utter certain elements of the vocal repertoire. The most notable effect was the persistent production of abnormal isolation peeps, including addition of new harmonic components. Two animals with large lesions of the amygdala failed to show alterations of vocalization.

In addition it was shown that bilateral lesions of the central tegmental tract of the midbrain, one of the largest and most ancient of cerebral pathways, led to a fragmentation of the trump display and eventually to its elimination.

Significance to Biomedical Research and the Program of the Institute: There is some evidence suggesting that in mammal-like reptiles (the antecedents of mammals) vocalization did not exist and, therefore, could not have played a role in social communication. It also seems probable that as in the case of most existing lizards, the mammal-like reptiles did not care for the young. With the evolution of mammals, it is evident that vocalization became of utmost importance in maintaining communication between mother and offspring and insuring against the possibility of separation. In this respect, the findings of alterations of the isolation peeps in the present experiments are of particular interest. Heretofore, according to one authority, alterations of vocalization (as opposed to mutism) have not been produced by cerebral lesions.

Proposed Course: To be continued.

Publications:

Jacobowitz, D.M. and MacLean, P.D.: A brainstem atlas of catecholaminergic neurons and serotonergic perikarya in a pygmy primate (Cebuella Pygmaea). Journal of Comparative Neurology, (in press).

MacLean, P.D.: On the evolution of three mentalities. In Arieti, S. and Chrzanowski, G. (Eds.), New Dimensions in Psychiatry: A World View, Volume II., New York, John Wiley & Sons, pp. 305-328, 1977. Also to appear in Washburn, S.L. and McCown, E.R. (Eds.), Perspectives on Human Evolution, Volume 4, Menlo Park, California, W.A. Benjamin/Staples Press, (in press).

MacLean, P.D.: The triune brain in conflict. In Bräutigam, W. and v. Rad, M. (Eds.), Psychotherapy and Psychosomatics, European Conference on Psychosomatic Research, Amsterdam, Basel, S. Karger, (in press).

MacLean, P.D.: Challenges of the Papez Heritage. In Livingston, K. (Ed.), The Continuing Evolution of the Limbic System Concept, New York, Plenum Publishing Company, (in press).

MacLean, P.D.: An evolutionary approach to brain research on "prosematic" (nonverbal) behavior. In Rosenblatt, J.R. and Komisaruk, B. (Eds.), Reproductive Behavior and Evolution, New York, Plenum Publishing Company, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00854-04 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ethogram of the Green Anolis Lizard, Anolis carolinensis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. B. Greenberg	Research Associate	LBEB NIMH
Other:	R. J. Radna	Research Associate	LBEB NIMH
	K. M. Sossin	COSTEP	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments on the green anolis lizard (Anolis carolinensis) are being conducted for testing the hypothesis that an ancient part of the forebrain referred to as the striatal complex plays a basic role in the organized expression of species-typical behavior in terrestrial vertebrates. For evaluating behavioral changes in experiments involving the paleostriatum and other parts of the forebrain, a detailed behavioral inventory and ethogram for this species is needed. This year's work has been largely devoted to a more detailed analysis of behavioral changes occurring in tests evoking the challenge display.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A primary purpose of this Laboratory is to test the hypothesis that the striatal complex plays a basic role in the organized expression of species-typical behavior of reptiles, birds, and mammals. For such comparative work it is essential to obtain a complete behavioral profile (ethogram) of the various species used in assessing the behavioral changes resulting from the experimental procedures. The present project is concerned with obtaining additional information about the ethogram of the green anolis lizard (A. carolinensis) that is being used in experiments on the paleostriatum.

Methods Employed: Observations of individual and social interactions are made on lizards living in compartmented enclosures separated by partitions that can be removed or substituted by transparent panels. Event recorders, television, cinematography, and computers are used for analyzing and quantifying the incidence of behavioral acts and states.

Major Findings: 1. In addition to the "arousal" ("signature"), "challenge", and "courtship" displays described in previous reports, a fourth display has been identified. Referred to as the "subordination" display, it has features of the "arousal" display, but occurs without the extension of the dewlap and the forelimbs.

2. Forty-seven behavioral subsets have now been identified as constituents of actions of the green anole during (1) maintenance of biological needs, (2) territorial defense and fighting, and (3) courtship.

3. In the preoperative tests of display behavior of male lizards it has been further learned that push-ups (with extension of throat fan), sagittal expansion, and engorged throat are the regularly occurring manifestations of the challenge display. In order to characterize the variability of a lizard's behavior under testing conditions, the data recorded in 500 sessions were analyzed: (1) subjects seem capable of individual recognition; (2) display by an antagonist male is not necessary to elicit a challenge response in the experimental subject; (3) tests in which an antagonist intrudes into a test subject's habitat are more effective in eliciting challenge responses than those providing only visual contact through a transparent divider; (4) dewlap extensions during challenge displays are often withheld when contenders are in close proximity; (5) the development of an eyespot, dorsal crest, and color changes of the body occur, respectively, about 270, 150 and 145 seconds following an initial challenge response; (6) the eye patch used for covering one eye of the experimental animal does not seem to interfere with the challenge response to an antagonist seen through the transparent panel; (7) lizards with eye patches are brown significantly more often than they are green; and (8) tongue touching occurs six times more frequently in strange than in home cages.

Significance to Biomedical Research and the Program of the Institute: The present project is intended to provide essential behavioral information about lizards used in the investigation of the evolutionary and neural roots of social communication.

Proposed Course: This project will be continued.

Publications:

Greenberg, N. B.: A neuroethological study of display behavior in the lizard Anolis carolinensis. Amer. zool. 17: 191-201, 1977.

Greenberg, N. B.: Social feeding in lizards. Herpetologica 32: 348-352, 1976.

Greenberg, N.B.: Ethological considerations in the experimental study of lizard behavior. In The Behavior and Neurology of Lizards, N.B. Greenberg and P.D. MacLean, eds., Rockville, Maryland, NIMH (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00855-03 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Stereotaxic Device and Brain Atlas for the Green Anolis Lizard (Anolis carolinensis)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. B. Greenberg	Research Associate	LBEB NIMH
	P. D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T. K. Bussard	Biologist	LBEB NIMH
	S. L. Thompson	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A stereotaxic brain atlas for the lizard Anolis carolinensis is being prepared for experiments involving stimulation or placement of lesions at selected sites in the brain. Thirty-four brains have been prepared for obtaining measures of variability and delineating nuclei and pathways.

Project Description:

Objectives: Observations made in this Laboratory indicate that the green anolis lizard (Anolis carolinensis) is a suitable reptile for comparative neuroethological studies (see reports M LBEB CN 30 and Z01 MH 00854-02 LBEB). For such work it is necessary to prepare a stereotaxic atlas of the brain.

Methods Employed: The stereotaxic technique was described in last year's report. A total of 34 brains have been prepared for learning the variation in coordinates of key structures and obtaining a clearer identification of cellular aggregates and associated fiber systems. For the latter purpose the cupric-silver method of de Olmos was used for tracing the small calibre fibers of the lateral olfactory tract after olfactory bulbectomy, and the Geneser-Jensen technique for revealing acetylcholinesterase was employed for differentiating paleostriatal structures.

Major Findings: For animals with a mean snout-vent length of 63.7 mm ± 2.6 (range 60-68 mm) the mean length of the telencephalon was 2.5 mm ± 0.3 (range 2.1-3.1 mm). The mean distance from the anterior commissure (AC) to the posterior commissure (PC) was 1.1 mm ± 0.2 (range 0.8-1.5 mm), while the mean distance from the AC to the frontal zero plane of the atlas was 0.9 mm ± 0.2 (range 0.7-1.2 mm).

The cupric-silver stain not only revealed the superficial distribution of the lateral olfactory tract, but also showed a distinct zone of terminals in the central area of the posterior dorsal ventricular ridge (PDVR). In this respect it is of interest to note that the arboreal green anole, as well as the knight anole (A. equestris) lack the nucleus sphericus, while the savannah dwelling brown anole (A. sagrei) possesses the nucleus.

The piriform area can be further delineated by a thin "pallial membrane" that thickens caudally to form a fiber-containing bridge from the piriform cortex to the DVR. A similar condensation of fibers is not seen rostral to the anterior commissure where the dorsal cortex fuses with the DVR.

As in turtles and birds, the stain for cholinesterase reveals that the paleostriatum is bounded dorsally by the dorsal medullary lamina.

Significance to Biomedical Research and the Program of the Institute: The preparation of this atlas is essential for neuroethological and neuroanatomical studies on the green anolis lizard (see report Z01 MH 00856-01 LBEB).

Proposed Course: To be terminated with publication of the atlas.

Publications:

Greenberg, N. B.: A neuroethological study of display behavior in the lizard Anolis carolinensis. Amer. zool. 17: 191-201, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-MH-00856-03 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Role of Paleostriatum in the Species-typical Display Behavior of Reptiles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. B. Greenberg	Research Associate	LBEB NIMH
	P. D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T. K. Bussard	Biologist	LBEB NIMH
	S. L. Thompson	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study on the green Anolis lizard (Anolis carolinensis) represents a further test of the hypothesis that the paleostriatum of reptiles, birds, and mammals plays a basic role in species-typical communicative behavior. It deals specifically with the effects of coagulative lesions of the paleostriatum on the display behavior of the green lizard. Such lesions may eliminate the challenge display, whereas similar destruction of other parts of the forebrain is without effect.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Previous work in this laboratory has shown that lesions of the pallidal part of the striatal complex may alter or eliminate the species-typical display behavior of squirrel monkeys (Z01 MH 00851-11 LBEB). The present comparative study on the green anolis lizard (Anolis carolinensis) is being conducted as a further test of the hypothesis that the paleostriatum plays a basic role in species-typical behavior.

Methods Employed: The method for testing pre- and postoperative display behavior of the green anolis lizard is described in report Z01 MH 00854-03 LBEB. Only vigorous and predictable displayers are used. Since the optic nerves are almost completely crossed, a lesion can be placed in one hemisphere and the animal's reactions to another lizard tested with a patch alternately placed on either eye. Microstereotaxic techniques are used to place unilateral coagulations in the paleostriatum and other parts of the forebrain. The intact hemisphere serves as a "control" for assessing both the behavioral changes and cerebral damage. Planimetry is used for obtaining volumetric measurements of the amount of cerebral destruction.

Major Findings: Experiments have now been completed on 16 lizards. Seven males with lesions of the paleostriatum showed significant deficits in their challenge display behavior. In all of these animals there was retention of the arousal display which may occur independently of social interaction (e.g., with change of perch). Animals with lesions of the dorsal ventricular ridge and one control in which an electrode was introduced without electrocoagulation showed no significant changes in the challenge display.

In all cases showing significant deficits in the challenge display there was damage to both the paleostriatum and to the lateral forebrain bundle (LFB), a major fiber bundle that connects the paleostriatum and other forebrain areas to the brain stem. Volumetric analysis showed that the amount of destruction to the paleostriatum ranged from as little as 1.4% to as much as 15%, a finding that suggests that failure of its functions or that of overlying structures could be attributed to a widespread loss of innervation because of damage to the LFB. In all cases degeneration was seen in both the ventral and dorsal components of the LFB that could be traced, respectively, to the nigral region and N. rotundus. No cell loss was seen in the latter nucleus.

Significance to Biomedical Research and the Program of the Institute: Experiments performed in this Laboratory on squirrel monkeys (Z01 MH 00851-12 LBEB) have provided evidence that the striatal complex plays a basic role in the organized expression of species-typical, prosematic behavior. Such behavior is employed in social communication by all terrestrial vertebrates including man. The present investigation provides further comparative evidence that the neural roots for such behavior can be phyletically traced to the paleostriatum.

Proposed Course: To be terminated with the publication of the results.

Publications:

Greenberg, N. B.: A neuroethological study of the display behavior in the lizard Anolis carolinensis. Amer. zool. 17: 191-201, 1977.

MacLean, P.D.: Why neuroethological research on lizards? In Greenberg, N. B. and MacLean, P. D., The Behavior and Neurology of Lizards, Rockville, Maryland, NIMH (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00860-03 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Unit Analysis of Vagal Inputs to the Amygdala, Hippocampus and Striatal Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Radna	Research Associate	LBEB NIMH
	P. D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T. K. Bussard	Biologist	LBEB NIMH
	A. H. Orr	Guest Worker	LBEB NIMH
	R. M. Sewell	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.4	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This continuing microelectrode study is concerned with the important question of the influence of interoceptive systems on unit activity of the forebrain. The experiments are performed on gently restrained sitting squirrel monkeys with chronically implanted electrodes next to the vagus nerve in the jugular fossa. This past year's work has provided a further characterization of units in the putamen and frontotemporal portion of the limbic system that are responsive to vagal volleys. Of particular interest was the finding of units in the limen insula, claustrum, anterior amygdala, and putamen that appeared to discharge in connection with respiration and that could be entrained by vagal volleys.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: There is little existing information regarding the important question of visceral influences on the three main evolutionary formations of the forebrain. Preceding experiments revealed that triple shocks applied to the vagus nerve elicited responses of single cells in the phylogenetically ancient intralaminar nuclei and in the limbic cortex of the cingulate gyrus. Exploration is now being extended to the hippocampus, amygdala, and putamen.

Methods Employed: Squirrel monkeys fitted with a chronically fixed stereotaxic platform are gently swaddled in a special chair during an experiment. Units are recorded extracellularly with glass micropipettes. Vagal shocks are applied with electrodes implanted in the jugular fossa. Control stimuli are applied through cervical subcutaneous electrodes. A flexible computer system (CAUDAR) utilizes wave-form recognition and provides high-speed graphic display of unit activity, surface plots of potentials of more than one unit, time-interval histograms, and a print-out of the statistical analysis.

Major Findings: Vagal volleys elicited responses in 17% of 86 hippocampal units; 11% of 97 amygdala units; and 31% of 99 units in the putamen. Significantly, almost half of the 13 cells in the central nucleus of the amygdala were responsive. For the total study, the number of initially excited and initially inhibited units was about equal. Most initially excited units responded with a burst of spikes. The shortest latencies were 80 msec for the hippocampus, 15 msec for the amygdala, and 14 msec for the putamen. A finding of unusual interest was the discovery of units in the anterior amygdala, limen insula, and putamen, that appeared to discharge in connection with respiration and could be entrained by vagal shocks.

Significance to Biomedical Research and the Program of the Institute: The vagally responsive units in the anterior amygdala and limen insula are located in areas known to be involved in olfaction and gustation. Although it might be expected that such neural elements would be implicated in reciprocal mechanisms of breathing and swallowing, the present experiments appear to provide the first evidence of a direct vagal influence on cells in this area. The implications with the respect to the respiratory-type neurons discovered in the putamen are not clear, but are of special interest in the light of respiratory tics described in diseases of the putamen and interruption of speech elicited by stimulation of the putamen.

Proposed Course: To be continued.

Publications:

Bachman, D.S., Allen, A. M., and MacLean, P.D.: Myocardial myocytolysis following vagal stimulation in squirrel monkey: a case report. Primates. 18 (2): 465-470, 1977.

Bachman, D.S., Hallowitz, R.A., and MacLean, P.D.: Effect of vagal volleys on serotonin on units of cingulate cortex in monkeys. Brain Research, 130: pp. 253-269, 1977.

Hallowitz, R.A. and MacLean, P.D.: Effect of vagal volleys on units of intralaminar and juxtalaminar thalamic nuclei in monkeys. Brain Research, 130: pp. 271-286, 1977.

Radna, R.J. and Vaughn, W.J.: Computer Assisted Unit Data Acquisition/Reduction, EEG Journal, (in press).

Radna, R.J. and Vaughn, W.J.: CAUDAR: Computer Assisted Unit Data Acquisition/Reduction, National Technical Information Service, Report Number NIH/DB-77/003, 1977.

Bachman, D.S., Hallowitz, R.A., and MacLean, P.D.: Effect of vagal volleys on serotonin on units of cingulate cortex in monkeys. Brain Research. 130: 253-269, 1977.

Hallowitz, R.A. and MacLean, P.D.: Effect of vagal volleys on units of intralaminar and juxtalaminar thalamic nuclei in monkeys. Brain Research. 130: 271-286, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00861-03 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Neuroethology of Reproductive Behavior: I. Role of the Accessory Olfactory System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Murphy Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Currently, the importance of the accessory olfactory system to the mating behavior of male hamsters is being tested. Following lesions of the vomeronasal nerves, 2 of 11 males at first failed to mate but recovered after 3 to 6 weeks. The other 9 males displayed subtle, but statistically significant, changes in mating behavior.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The accessory olfactory system, or vomeronasal system, is present in most terrestrial vertebrates; yet its specific functions remain unknown. Traditionally, it has been regarded as a mere supplement to the main olfactory system. The recent discovery that the central neuroanatomical projections of the accessory olfactory bulb are distinct from those of the main olfactory bulb has indicated that the vomeronasal system may have an independent behavioral significance. In previous studies sections of the vomeronasal nerve have been found to have a dichotomous effect on the mating behavior of male hamsters, leaving some animals unaffected and others deprived of the ability to copulate. The purpose of the current project is to determine if any aspect of mating behavior is consistently affected by lesions of the vomeronasal nerve.

Methods Employed: Subjects were 21 sexually experienced male hamsters, descended from stock captured in Syria in 1971. The vomeronasal nerves were sectioned in 11 subjects; the remainder served as controls. Following recovery from surgery each male was observed for 30 minutes with an estrous female hamster. Sixteen behavior patterns were recorded on a computerized event recorder and analyzed with respect to frequency, total duration, latency and average duration of each activity.

Major Findings: Nine of the experimental and all of the control subjects mated and achieved at least one ejaculation. The analysis revealed several statistically significant subtle changes in the mating behavior of the males deprived of vomeronasal innervation, including a reduced frequency of grooming and an increased latency to the first intromission. The two operated males that originally failed to mate recovered normal performance 3 to 6 weeks later. Microscopic examination of the lesions has not yet been completed.

Significance to Biomedical Research and the Program of the Institute: The present study has shown that section of the vomeronasal nerves results in subtle changes of mating behavior of male hamsters, but as opposed to the findings of Winans and Powers, does not eliminate mating. One possible explanation for the difference in the two studies is that the hamsters used in the present experiments were bred from recently wild stock.

Proposed Course: To be continued.

Publications:

Murphy, M.R.: Olfactory impairment, olfactory bulb removal and mammalian reproduction. In "Mammalian Olfaction, Reproductive Processes, and Behavior". Ed. R.L. Doty, Academic Press, N.Y. 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00863-03 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Social Preference Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Murphy Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Neural and other factors which regulate species identification and social preferences are being investigated. Male hamsters of two different species are used as subjects. In Experiment 1 it was found that cross-fostering by a female or another species eliminated or weakened, but did not reverse adult sexual preferences. In Experiment 2 simultaneous destruction of both the main and accessory olfactory bulbs completely eliminated the preference for conspecific females; however, lesions of the sensory nerve supplying the accessory olfactory system had no effect on species preferences.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project deals with the fundamental question of what neural and other factors are critical for mammals of a particular species to recognize and mate with their own kind. The first of two experiments on hamsters examines the influence of early experience. In the second experiment an analysis is made of the role of olfaction. The recent discovery that the central neuroanatomical projections of the accessory olfactory bulb are distinct from those of the main olfactory bulb has suggested that the behavioral functions of these two aspects of olfaction may also be distinct. Therefore, in this study attention is focused on the effects of lesions of the accessory olfactory system.

Methods Employed: In Experiment I Syrian and Turkish hamster males were respectively cross-fostered by a lactating female of the other species. Members of the control groups were raised by lactating females of their own species. Upon reaching maturity, the sexual preference of all males was tested by placing them individually for ten minutes with two estrous female hamsters, one Turkish and one Syrian. The females were anesthetized so that their behavior or vocalizations would not influence the male's choice. The behavior of the males was recorded and analyzed with a computerized event recorder.

In Experiment II the vomeronasal nerves supplying the accessory olfactory system were sectioned in 16 male Syrian hamsters. Ten males first serving as control subjects were later subjected to removal of the olfactory bulbs, an operation that eliminated input to both the main and accessory olfactory systems. Following recovery and an opportunity to mate with an estrous female of their own species, the sexual preference of each male was tested.

Major Findings: Experiment I. Rearing by a female of a different species eliminated a preference for conspecific females in Syrian males and weakened conspecific preference in Turkish males.

Experiment I. Simultaneous destruction of both the main and accessory olfaction bulbs completely eliminated the preference for conspecific females; however, section of the sensory nerve supplying the accessory olfactory system had no effect on species preferences. Microscopic examination of the lesions is not yet complete.

Significance to Biomedical Research and the Program of the Institute: The finding of Montagner that children can discriminate a blouse worn by their own mother demonstrates an importance of olfaction in the development of human social behavior. The results of the current project indicate that in male hamsters the tendency to mate with females of the same species is based on an olfactory preference which is in part acquired during early development.

Proposed Course: This study will be continued.

Publications:

Murphy, M.R.: Intraspecific sexual preferences of female hamsters. Journal of Comparative and Physiological Psychology, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00864-03 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of the Biogenic Amines in Aggressive and Reproductive Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Murphy Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Currently, this project involves studies on the interactive effects of stimulation of the dopamine system and impairment of the olfactory system on the social behavior of male hamsters. It has been found that apomorphine, a putative activator of dopamine receptors, greatly inhibits copulatory behavior and increases aggressive behavior in sexually active male hamsters. Apomorphine also increases aggression in olfactory bulbectomized males, indicating that the drug acts independently of the normal olfactory sensory control of aggression in hamsters. The only animals failing to show a response to apomorphine were four males in which lesions of the sensory nerve supplying the accessory olfactory bulbs had temporarily eliminated mating behavior.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Dopamine receptors are concentrated in the striatal complex of the forebrain. There are reports in the literature that apomorphine, a putative activator of dopamine receptors, has a facilitating effect on both mating behavior and aggression in male Syrian hamsters. Since the normal initiation of either mating or aggression in hamsters depends on the sense of olfaction, it is significant to learn whether or not apomorphine exerts its effect by action on olfactory structures. The purpose of the present project is to test the effects of simultaneous stimulation of the dopamine system and impairment of the olfactory system on mating and aggressive behavior of hamsters, eliminating the entire olfactory apparatus in some animals, and only the accessory system in others.

Methods Employed: Twenty-six sexually experienced male hamsters were used. Ten males, serving first as control subjects, were later subjected to a bilateral olfactory bulbectomy. In the 16 other males the vomeronasal nerves supplying the accessory olfactory bulb were cut; five of them were sexually inexperienced at the time of the surgery. Tests involved observing a male with an estrous female hamster for 30 minutes. Fifteen behavior patterns were recorded and analyzed with a computerized event recorder. All animals were first tested under the influence of apomorphine (0.3-0.6 mg/kg). After recovery from surgery they were tested following a control injection of physiological saline. Five to 15 days later they were again tested under the influence of apomorphine. Tests were begun immediately after injections.

Major Findings: In the control group receiving apomorphine, there was a marked increase of aggression and an inhibition of mating. The amount of aggression or mating was not significantly different in the two tests with the drug, indicating that apomorphine did not have a persisting effect on behavior. In olfactory bulbectomized males treated with apomorphine there was also a high level of aggression indicating that such pharmacologically induced aggression occurs independently of the normal olfactory control of aggression. Twelve of the 16 males with section of the vomeronasal nerve mated following recovery from surgery; these animals responded to apomorphine in the same manner as the controls. However, the 4 males that temporarily failed to mate following vomeronasal lesions, also failed to display a significant response to apomorphine.

Significance to Biomedical Research and the Program of the Institute: The present results provide evidence that the dopamine system may act independently of olfactory influences in the elicitation of aggressive and reproductive behavior. Since one component of the mesolimbic dopamine system projects to the olfactory tubercle, the findings suggest an interaction of olfactory and dopamine systems in their influence on social behavior.

Proposed Course: To be continued.

Publications:

Murphy, M.R.: Blinding increases territorial aggression in male Syrian golden hamsters. Behavioral Biology 17: 139-141, 1976.

Murphy, M.R.: Olfactory stimulation and olfactory bulb removal: Effects on territorial aggression in male Syrian golden hamsters. Brain Research, 113: 95-110, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00868-02 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Time Series Analysis of Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. T. Nunez Psychologist LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with the problem of obtaining accurate and sensitive descriptions of sequences of behavior in time. Traditional behavioral studies have seldom dealt with these questions. In the present study methods of time series analysis are used to quantify and describe behavioral patterns. Ethological observations are made using an event-recorder program run on a laboratory computer. Analysis includes the evaluation of correlograms and power spectra. A comparison of the mating behavior of two species of hamsters has revealed a different pattern in the occurrence of several components of behavior.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project applies advanced methods of statistical analysis to the study of animal behavior. The research effort is divided into two interrelated parts. (1) the development and evaluation of time series analysis suitable for characterization of the behavior of animals observed in a natural or free-running situation; and (2) the application of these methods to questions of the neural and adaptive significance of behavior. A comparison of the sexual behavior of two species of hamsters was made.

Methods Employed: In addition to the usual methods of behavioral analysis, the study employs time series analysis, which provides greater information about the time-dependent aspects of a sequence than do totals of the number of events in a sequence. Specifically, each data-record is used to construct correlograms, both auto and cross, and coherence plots. The correlograms are transformed into power spectra for frequency analysis. These tests are used in conjunction with the more standard group comparisons of frequencies and durations of behavioral events.

A comparison was made of the normal species-typical sexual behavior of two species of hamster (Syrian: Mesocricetus auratus, and Turkish: Mesocricetus brandti). Both groups tested were descended from stock recently captured in the wild.

A software event recorder, run on a Wang 2200 calculator, stored the observer's record of the animal's behavior for transmission to the NIH Computer Center (DCRT), where BMD library programs produce the plots and spectra.

Major Findings: There were few significant differences between the total frequencies of sexual and non-sexual behavior of the two species. In sexual sequences, both species employed the same components of behavior. However, the timing of these components was different for Syrian and Turkish hamsters. For example, the number of mounts and intromissions for the two groups were about the same, but their patterns of occurrence were different, with the Syrians showing regularity in timing that lasted further into their sessions. Statistically, it does not seem possible to compare directly individual time series results, but the plots of typical animals make the difference clear. Given the wide variability within the species' groups, it is doubtful that the observed differences are in themselves sufficient to make sexual behavior an isolating mechanism for these sympatric species; but such differences might raise the threshold of interspecific response (demonstrated in this Laboratory by preference studies), high enough to preclude successful cross-breeding.

Significance to Biomedical Research and the Program of the Institute: The methods of analysis and data recording developed for this project are relatively new to the field of animal behavior, allowing a detailed and accurate examination of the organization of behavior.

Proposed Course: This project will be completed with the analysis of the mating behavior of the micrencephalic hamsters described in Project Z01 MH 00870-01 LBEB. A description of the methods and findings will form the major part of a doctoral thesis in psychology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00869-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Striatal Complex: Question of Role of Dopamine and Opiate Receptors in Species-typical Behavior of Canids.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. F. Bacon	Research Psychologist	LBEB, NIMH
	P. D. MacLean	Chief, LBEB	LBEB, NIMH
	M. R. Murphy	Senior Staff Fellow	LBEB, NIMH
	J. M. Stewart	Research Psychologist, URBS	LBEB, NIMH
Other:	R. M. Sewell	Biologist	LBEB, NIMH

COOPERATING UNITS (if any)

W. Bunney, A. Pert, C. Pert, G. Davis, NIMH, Adult Psychiatry Branch, Section on Neuropsychopharmacology
E. Baas, DRS, VRB, Animal Center Section, Carnivore Unit

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837 2.1

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	0.8	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In a neuropharmacological study on the functions of the striatal complex, observations have been made on the effects of neuropharmacological blockers of dopamine and of opiate receptors on the development of species-typical behavior of canids. Pimozide-treated beagle dogs failed to develop several behavioral characteristics of the breed. Naltrexone-treated dogs were not remarkable except for some aberrations pertaining to sexual behavior.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A primary purpose of this Laboratory is to test the hypothesis that striatal complex (an evolutionary ancient part of the forebrain) plays a basic role in the organized expression of species-typical behavior. Parts of the striatal complex are rich in dopamine and opiate receptors. Consequently, it would be significant in regard to striatal function to learn whether or not the chronic administration of pharmacological blockers of dopamine and opiate receptors would affect the unfolding of species-typical behavior in developing animals. In other words, would it affect the way in which they "read their environment." Dogs have been used as trial subjects in this and other work on the striatal complex because they exhibit not only species-typical behavior, but also breed-typical behavior.

Methods Employed: Pimozide and Naltrexone were selected as pharmacological blockers, respectively, of dopamine and opiate receptors. Half of one litter of beagle puppies was treated with the respective drug beginning at about 2 weeks of age; the other half served as control subjects. Drug treatment continued for a period of 9 months. Adequate dosage was periodically confirmed by challenges with apomorphine and with methadone. In addition to ethological observations, a battery of tests were given at specified intervals. The tests included: (1) vocal response to isolation; (2) leash training; (3) response to an active and passive handler; (4) competition over a bone; (5) response to a strange dog in the pen; (6) performance in the field; (7) sexual response of males to females in heat.

Major Findings: The pimozide-treated animals developed many behavioral aberrations. Specifically, they (1) vocalized significantly more ($p<.05$) in the isolation tests than the control animals; (2) did not learn to walk on the leash; (3) did not engage in usual allelomimetic behavior; (4) drooped awkwardly instead of cradling and seeking the handler's face when being held; (5) inappropriately rolled over in a submissive posture when approached by handlers (males did same in response to approach by a female in estrus); (6) showed unusual aggression towards littermates; (7) failed to show the trail following responses of beagles in the field.

Significantly, in challenging the pimozide-treated dogs with increasing doses of apomorphine, they proved to be protected against the emetic effects, but there was a marked aggravation of stereotypic behavior.

The naltrexone-treated dogs and the control littermates showed no notable deficits in the above listed tests. The treated animals, however, displayed unusual behavior for beagles insofar as one male precociously showed continual mounting behavior, while two females became uncontrollably aggressive, directing their attack at the genital region of the control females.

Pilot observations were made on the chronic administration of methadone to dogs under the same conditions described above. Although the dose of the drug was such that the animals did not show signs of addiction, there were several significant findings such as reduced vocalization in isolation and a failure of tests in the field.

Significance to Biomedical Research and the Program of the Institute: The present study illustrates that in neuropharmacological investigations on cerebral functions, ethological observations may uncover behavioral aberrations that would not be seen in conventional tests. Of particular interest was the failure of the pimozide-treated dogs to develop several behavioral characteristics of their breed. Neuropharmacologically, their paradoxical response to apomorphine deserves further analysis.

Proposed Course: To be completed with the publication of the results.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-MH-00870-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Neuroethological Study of Micrencephalic Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. R. Murphy	Senior Staff Fellow	LBEB NIMH
	P. D. MacLean	Chief, LBEB	LBEB NIMH
Other:	S. C. Hamilton	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.4	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an investigation of functions of the striatal complex and the limbic system, ethological observations have been made on the development of hamsters deprived since birth of the neocortex. Such preparations grew and developed like normal animals, showing the usual species-typical behavior, including successful mating and breeding.

PHS-6040

Project Description:

Objectives: Laqueur et al produced micrencephaly in rats through the necrotizing effect of methylazoxymethanol (MAM) on the matrix cells of the neocortex on the 14th and 15th days of gestation. These animals devoid of neocortex and part of the limbic cortex scored almost as well as normal animals in a battery of psychological tests and were successful in mating and rearing young. Since a primary concern of our Laboratory is to investigate the role of the reptolimbic formations of the forebrain in species-typical behavior and motivation, such micrencephalic preparations provide unusual opportunities for behavioral and neurological research. The purpose of the present project is to examine the capabilities of micrencephalic hamsters in the organized expression of species-typical behavior.

Methods Employed: In order to avoid other complicating effects of a drug such as MAM, thermal destruction of the developing neocortex was accomplished on the first postnatal day. Each experimental litter was matched with a normal one of identical size and age. The development of these animals was followed with respect to an ethogram comprising more than 30 behavioral constructs. In addition, special tests were conducted on thermotaxis, nest building, burrow activity, homing, hoarding, retrieval, species identification, and mating. Time lapse television was used to monitor the diurnal activity.

Major Findings: Two litters of micrencephalic hamsters have been observed. Histological examination of the brains has not yet been completed. In key animals there was absence of the neocortex, a massive reduction of the neothalamus, and no pyramidal tracts.

Hamsters deprived from birth of neocortex grew and developed like normal animals. They showed usual hamster-typical behaviors, including playing, flank marking, nest building, hoarding, establishing a urination post, fear reactions, and characteristic diurnal activity. A female with symmetrical absence of the neocortex mated and reared her young. Although having some motor disability, the male hamsters mated and sired normal young.

Finally, the responses to neuropharmacological agents were similar to those of normal hamsters. Males, treated with apomorphine, for example, showed inhibition of mating and increased aggression.

Significance to Biomedical Research and the Program of the Institute: The ethological observations and formal tests conducted on hamsters developing from birth without neocortex support the view that the striatal complex and limbic system have the capacity to give expression to the entire range behavior involved in survival of the individual and the species.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00871-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparative Histochemical Analysis of the Ventral Striatal Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.C. Switzer, III Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (If any)

NONE

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Laboratory of Brain Evolution and Behavior

SECTION

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.55	0.35	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A primary purpose of this Laboratory is to test the hypothesis that the striatal complex plays a basic role in organizing the expression of species-typical behavior. Anatomical and histochemical findings suggest that some of the large neurons of the interior zone of olfactory tubercle (ventral striatum) may be a rostroventral extension of the globus pallidus. Accordingly, in rats the large cells of the tubercle are being compared with those of the globus pallidus with respect to their content of glutamic acid decarboxylase (GAD) (the synthesizing enzyme for gamma amino butyric acid [GABA]) and of ferritin. Both ferritin and GAD can be localized by immunochemical methods. Comparative observations are also to be made on lizards.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: It has been hypothesized that the polymorph layer of olfactory tubercle in rat is a rostroventral extension of the globus pallidus. The unique chemistry of pallidal neurons allow a test of this hypothesis. In this project the large motor-like neurons of the tubercle are to be compared with those of globus pallidus for their content of glutamate decarboxylase (GAD) and of ferritin. As a preliminary to extending the observations to reptiles, a stain for acetylcholinesterase is being used to identify the striatal complex in lizards.

Methods Employed: Both histochemical and immunochemical procedures may be used for localizing GAD and ferritin. Since iron is stored in pallidal neurons as ferritin in greater quantities than in surrounding cells, the presence of ferritin will serve as an indicator of pallidal character for the large motor-like neurons of olfactory tubercle. To maximize the presence of ferritin, the rats to be examined are given an iron-enriched diet. A modification of the Koelle method is used for demonstrating the distribution of acetylcholinesterase in the lizard.

Major Findings: The experiments concerned with ferritin have just begun. As in the turtle, the stain for cholinesterase has shown that the striatal complex in the lizard (Anolis carolinensis) lies below the dorsal medullary lamina.

Significance: It is anticipated that the criteria applied in this study will be useful in determining the rostroventral limits of the globus pallidus in mammals and lizards. A further impetus to obtain a more complete characterization of the striatal complex stems from the recent demonstration of estrogen and opiate receptors in ventral striatum, raising the question of their involvement in the processing of exteroceptive and interoceptive inputs unique to this evolutionary ancient part of the forebrain.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00872-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparative Study of Olfactory Projections to Neopallium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.C. Switzer, III Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	.40	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the opossum the olfactory bulb projects directly to the neopallial part of the insula, while in rat the piriform cortex provides an olfactory input to a corresponding area. The insular area in question is adjacent to the cortical area representing taste. The present study has been undertaken to investigate olfactory projections to the insula in the squirrel monkey.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: In a preceding study it was found in the opossum that the lateral olfactory tract projects to the neocortical part of the insular cortex. In the rat, however, it was shown that no such direct pathway exists, the olfactory input being by way of the piriform cortex. Unpublished data of Heimer indicate that a similar condition exists in the rhesus monkey. Since the olfactory projections have never been mapped in a New World monkey, experiments are being performed on squirrel monkeys in order to learn whether or not there are olfactory connections with the insula in this species.

Methods Employed: The olfactory bulb was removed unilaterally in a squirrel monkey. After a survival time of four days the animal was perfused and the brain removed and prepared for cutting frozen sections. The sections were processed for showing neural degeneration with the cupric-silver method of de Olmos.

Major Findings: A direct projection to the limen insula of squirrel monkey was not observed. Since a shorter survival time may prove more satisfactory for showing terminals, other animals must be prepared in which the survival time will be two days. Pending that outcome, lesions will be placed in the piriform cortex.

The present material revealed an olfactory projection to the olfactory tubercle that was more medial than previously seen in primates. The terminal degeneration was medial to the layer of packed cell bodies forming layer II of the piriform cortex and located in pockets adjacent to the densely p acked clusters of dwarf cells in the islands of Calleja. Heretofore, the presence of a tightly packed layer of cells as a continuation of layer II of the piriform cortex was considered necessary for the existence of olfactory input, an assumption that is now no longer tenable. This evidence strengthens the arguments that the olfactory tubercle is a specialized zone of ventral striatum.

A further analysis of olfactory projections to the thalamus and cortex in the rat resulted in the following conclusions:

A. The neocortex receives an olfactory input by at least two routes;

   1. via the medial dorsal thalamus
   2. directly from the piriform cortex

B. The medial dorsal nucleus of the thalamus (a major source of input to the frontal cortex) also receives olfactory inputs from multiple sources:

   1. olfactory tubercle
   2. amygdala
   3. piriform cortex
   4. insular cortex (as a target of piriform cortex efferents)

Significance to Biomedical Research and the Program of the Institute: The unexpected finding of an olfactory projection to the ventral striatum in the squirrel monkey may have particular significance since the projection is to a zone closely associated with the islands of Calleja, which have a high affinity for estrogen and possibly opiates. Embedded within the islands are large motor-like neurons, some of which project to the mediodorsal nucleus (MD). Since MD projects to frontal cortex, there exists a possible mechanism by which estrogen and opiates could affect one of the most highly evolved parts of the brain.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00873-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Taxonomic Classification of Mammals Based on the Trajectory of Dorsal Lateral Olfactory Tract

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.C. Switzer, III Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	.20	0.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This comparative study was completed with the examination of the trajectory of the dorsal component of the lateral olfactory tract (dLOT) through the accessory olfactory formation in squirrel monkey. A review of over 40 mammals revealed that in some species the dLOT runs between the large output neurons of the accessory olfactory formation and the internal granule cells, while in others it passes below the internal granule cells. A grouping of the animals according to this criterion leads to the same classification of placental mammals as a taxonomy based on bones and teeth. In carnivores and ungulates (both odd and even toed hoofed animals) the dLOT passes below the internal granule cells, while in rodents, edentates, lagomorphs, insectivores and primates, the dLOT separates the internal granule cells from the large output neurons. The results demonstrate that comparative studies of brain structures provide inferences regarding the ancestry of the orders of mammals.

Project Description:

Objectives: More than 40 mammalian species had been previously examined for testing the hypothesis that in animals derived from leptictid-like insectivora, the dorsal lateral olfactory tract (dLOT) passes between the large output neurons of the accessory olfactory formation and the internal granule cells, whereas in animals derived from palaeoryctid insectivora it courses below the internal granule cells. Since no New World monkey had yet been examined in this respect, it was important to learn whether the hypothesis applied to a representative New World primate such as the squirrel monkey.

Methods Employed: The olfactory bulb was removed on one side of a squirrel monkey (sparing the accessory formation) for the purpose of tracing the degeneration of the dLOT with the cupric-silver method. Alternate sections were stained for Nissl substance so as to confirm the existence and disposition of the cell bodies of the accessory olfactory formation.

Major Findings: On the basis of the trajectory of the dLOT through the accessory olfactory formation (AOF) the orders of placental mammals thus far studied have been grouped as follows:

dLOT dividing AOF	dLOT below AOF
Lagomorpha	Carnivora
Edentata	Hyracoidea (Hyrax)
Rodentia	Perissodactyla (e.g., zebra)
Insectivora	Artiodactyla (e.g., sheep)
Dermoptera	
Primates	

In line with the above classification, the trajectory of the dLOT in the squirrel monkey was the same as in other primates.

Significance to Biomedical Research and the Program of the Institute: The conspicuous, yet subtle difference in the trajectory of the dLOT through two segregated populations of cells in the same formation provides another kind of evidence that supports a division of the placental mammals according to ancestral derivation. The present study also provides cogent evidence that differences in brain organization of living animals is useful for taxonomic purposes. It is implicit in these observations that there is an invariant expression of genetic factors controlling the time of axon growth (i.e., dLOT) and of migration of cell bodies (the internal granule cells) in a group of animals with highly diverse phenotypes.

Proposed Course: This study is completed and ready for publication. It forms the first part of a series of three articles; the other two deal with a taxonomic analysis based on neuroanatomical differences.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00874-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Neuroethology of Reproductive Behavior: II. Question of Role of Endogenous Opiates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Murphy Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate peptides and opiate receptors are concentrated in parts of the limbic system and striatal complex. It has been suggested that endogenous opiates may be involved in sexual behavior. This possibility was tested by observing the effects of the opiate antagonist naltrexone on the mating behavior of male hamsters. A detailed ethological analysis revealed several aberrations in the mating behavior of naltrexone-treated animals. In general, their behavior appeared sluggish and inattentive. They were capable, however, of successfully impregnating females.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Opiate peptides and opiate receptors are concentrated in parts of the limbic system and striatal complex. It has been suggested that endogenous opiates may be involved in sexual behavior. The aim of the present project is to investigate this possibility with respect to mating behavior.

Methods Employed: Forty sexually inexperienced male hamsters were each given two tests of mating behavior. In order to block the action of endogenous opiates, large doses (20 mg/kg) of the opiate antagonist naltrexone were administered; physiological saline was given as a control injection. In the first test 20 males received naltrexone and 20 received saline; in the second test half of the members of each of the original groups received the drug and half received saline. Sixteen behavioral patterns were recorded on a computerized event recorder and analyzed with respect to frequency, total duration, latency and average duration of each activity.

Major Findings: The effects of naltrexone on behavior were much greater in sexually experienced males than in sexually inexperienced males, in part because sexually experienced males mated like animals without experience. In general, naltrexone treated animals were slower to start mating; spent less time copulating and more time ignoring the female than did controls. Animals receiving naltrexone took longer to resume mating following an ejaculation. There was a longer average duration per activity for several aspects of behavior. Their mating thus might be described as sluggish and inattentive.

In spite of the many deficits in their mating behavior, the naltrexone treated animals successfully impregnated females and their offspring developed normally.

Significance to Biomedical Research and the Program of the Institute: The results are compatible with the proposal that the endogenous opiates play a role in sexual behavior. This experiment illustrates that a detailed ethological analysis may uncover behavioral changes that might not be apparent with the use of conventional tests.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00832-01 LBEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Designing Built Environments to Promote Behavioral Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	J. H. Burkholder	Research Psychologist	LBEB NIMH
	N. A. Slade	Intergovernmental Exchange Fellow	LBEB NIMH

COOPERATING UNITS (if any)
none

LAB/BRANCH
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SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, LBEB, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Designed environments are built to house groups or populations of house mice or Norway rats. Through the locations of resources, response eliciting opportunities, partial barriers and travel routes, environments are designed to influence: choice, frequency of social interaction, cooperation, privacy, group integrity, formation of communication networks, identity differentiation, and perceptual and motor skills contributing to coping and creativity. Such influences on behavior are realized by instituting an unambiguous environmental language through spatial and structural relationships of components and by uniqueness of texture and form related to opportunity to express each specific behavior or set of related behaviors. In essence this project represents behavior related to experimental architecture. Influences upon behavior and social organization of environmental design are assessed through strategies of inquiry incorporated in projects Z01 MH 00833 to 00836, Emphasis in this project is placed on the behavioral zoögraphy (spatial distribution of behaviors) and upon the expression of territoriality. The scope of this project encompasses aspects of previous projects Z01 MH 00823, 24, and 29.

Project Description:

Objective: To determine the principles whereby the characteristics of experimentally designed, built environments promote health, behavioral adequacy, and adaptive capacity of rodents.

Methods Employed: Two types of environments have been built to house populations of 100 to 1000 individuals. One is radially symmetrical and the other bilaterally symmetrical. A third, triangularly symmetrical, provides the minimal space and structure to study parental care of subjects removed from the larger environments. Locations in each environment are minutely coded. A mnemonic language for rapid coding of behavior and animal identity has been developed for manual recording or direct input to a computer utilizing an electronically sensitive x-y grid providing means for rapid recording of identity, location, time and behavior. In addition, the bilaterally symmetrical environment incorporates technology for continuously recording animal identity, place, time, and direction of travel and behavior of every member of the population.

Major Findings: Results here reported are confined to one population of mice inhabiting a radially symmetrical environment. The incorporated, advanced design criteria, encompassing eight cells or regions of identical structural and spatial composition, has permitted the population to attain a state of optimum density at which each cell houses an optimum-sized group based on data relating to (1) stability of group membership and relationships, (2) ability to rear young effectively, and (3) moderation of intragroup and intergroup conflicts. Each member of each group exhibits a unique, differential usage of available places. This uniqueness of space utilization is so closely associated with prior behavioral and social history that knowledge alone of how a mouse distributes its activities over space suffices to indicate the major characteristics of its behavioral and social history. Provision of a variety of substrates, items and their locations, relating to nest-building has permitted definition of variables such as lineage, age, and developmental experience -- particularly social -- which influence the capacity of individuals to express inbuilt behavioral tendencies or to elaborate them into truly creative adaptations.

Significance to Biomedical Research and the Program of the Institute: The amount of existing built physical environment utilized by people in the United States has been conservatively estimated at a construction cost of $1,800 per person per year. We do spend most of our lives in human-designed and built structures. And yet our knowledge of how this built environment affects the health and behavior of people is very meager. However, since such structures have an average lifetime in excess of 20 years, knowledge of their influence can serve as a major means of implementing preventive and promotive mental health. The present project represents one of a very few, if not the only, experimental efforts with animals to reveal relevant design principles.

Proposed Course: The behavior and health of mice and rats inhabiting these three types of designed habitats will continue to be studied over the next three years.

Publications:

Calhoun, J. B.: "Looking Backward" from the beautiful ones. In W. R. Klemm (Ed.): Discovery Processes in Modern Biology, Huntington, N.Y., Robert E. Krieger Publishing Co., 1977, pp. 26-65.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00833-01 LBEB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Behavioral and Physical Development in Complex Environments.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. H. Burkholder	Research Psychologist	LBEB NIMH
Other:	W. M. Baum	Research Psychologist	LBEB NIMH
	J. B. Calhoun	Research Psychologist	LBEB NIMH
	J. M. Stewart	Research Psychologist	LBEB NIMH

COOPERATING UNITS (if any)
none

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

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INSTITUTE AND LOCATION
NIMH, LBEB, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	1.6	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physical and behavioral development of rodents were studied in the following ways: (a) 15 pairs of mice, each of which had siblings of comparable history remaining in a complex environment, were removed from this environment shortly after they attained reproductive capacity. Parental care-giving behavior of each of these pairs was studied in a small environment designed for this purpose. (b) Strains of wild Norway rats were screened for resistance to an anti-coagulant drug (Warfarin) to enlarge our stock of Norway rats suitable for pursuing developmental and other behavioral research. (c) Nest-building behavior of mice was recorded with respect to age, and social history. (d) As a part of a broader effort to develop a "catastrophe theory" formulation of behavioral states, motivation, drive, emotion, and mood. Focus was placed on two types of inter-response times characterizing lever pressing for water. (e) Circadian rhythms of mice by social history type and by type of behavior are being studied. This project includes parts of discontinued projects Z01 MH 0025, 26, and 27.

Project Description:

Objectives: To determine the manner in which physical environmental structure, genotype, group and population size, genealogical history, and opportunity for adaptation to changed circumstances influence behavioral and physical development from birth to death.

Methods Employed:

(a) Over a two-hour period each day until weaning at 21 days, the behavior of mother, father, and litter members is recorded with respect to the influence of caregiving behavior of parents on the development of their progeny.

(b) A six-day test on consumption of the anticoagulant drug, Warfarin, on 3 strains of wild rats was conducted.

(c) During weekly examinations of nest boxes for litter condition, detailed notes are maintained on the utilization of three types of nesting material in nest construction.

(d) Every lever press for water by 16 rats over a period of several months was recorded with time of press recorded to the nearest 0.1 second. Inter-response times were then calculated and curves of frequency as a function of inter-response interval were plotted.

(e) Based on hand recorded data encompassing 30,000 mouse hours of observation, a computer tape has been formatted for studying circadian rhythms of activity by individual and behavior types.

Major Findings: Those related to individual development include: (a) None, data collection on parental behavior of parents born at sub-optimal densities has just been completed. (b) Of the three strains of wild Norway rats tested, only one proved to be resistant to the anti-coagulant drug, Warfarin. We have retained the non-resistant Parsons Island Wild (PIW) and the anti-coagulant resistant strain from New York (ACR/N) for behavioral studies. This latter, based on experience in routine handling of them, has a very low threshhold for elicitation of aggressive behavior; they are so reactive that investigators have frequently been bitten. (c) The full capacity for nest building behavior has been very slow to unfold. The second generation, born in the complex habitat, essentially used only one of three nest-building materials. Then only by the time of the third litters born in the habitat were all materials used in a meaningful pattern to enhance survival of litters. (d) Lever pressing, which does deliver water, is executed at two different rhythms, one with a mean inter-response time of 1.5 seconds and the other at nearly five times that duration (i.e., 7.5 seconds). This finding of a 1.5 ratio has provided an essential clue to confirmation of a "catastrophe theory" formulation of behavioral states that all behavior must fit into a double octave sequence demanding that all time of any mammal must be apportioned into 16 categories regardless of evolved complexity. (d) Analyses are

just beginning of circadian rhythms employing the data base of 30,000 mouse hours of observation of behavior.

Significance to Biomedical Research and the Program of the Institute: Experimental studies of individual development have over recent years increasingly dwelt upon ever smaller segments of influence upon development as well as focused upon fewer consequences of experience. Our studies on individual development as a consequence of experience in complex physical and social settings, promises to restore a balance to inquiry so that molecular and holistic research may more likely provide insights applicable to both animals and humans exposed to complex, varying environments. In particular our efforts directed at establishing a general theory of behavioral states may provide the basis for guiding development and designing therapy.

Proposed Course: (a) Parental care will be studied over the next two generations after population density reaches 2 and 4 times optimum. (b) No more studies on anti-coagulant resistance are planned. (c) Capacity to construct adequate nests will be monitored with the anticipation that the capacity to express such integrative behavior will decline with increasing population density. (d) Our double-octave theory of behavioral states should be relatively complete by the end of another year. (e) Analyses of circadian rhythms will be continued.

Publications:

Calhoun, J. B.: Social modification of activity rhythm in rodents. In Proceedings, Twelfth International Conference - International Society for Chronobiology, Washington, August 10 - 13, 1975. Pp. 83-93. Publishing House Il Ponte, Milano, Italy. 1977. 765 pp. In press.

Baum, W. M.: Matching, Undermatching, and Overmatching in Studies of Choice. Journal of the Experimental Analysis of Behavior. In press.

Stewart, J. M., Scott, J. P., and DeGhett, V. J.: The Development of Social Affects: Effects of early experience on separation distress. Revista Latino-Americana-de Psycologia. 9:(2): 247-257, 1977.

Rose, R. K., N. A. Slade and J. H. Honacki. 1977. Live Trap Preferance Among Grassland Mammals. Acta Theorologica. 22:--. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00834-01 LBEB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Social Group Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. H. Burkholder	Research Psychologist	LBEB NIMH
Other:	J. B. Calhoun	Research Psychologist	LBEB NIMH
	W. M. Baum	Research Psychologist	LBEB NIMH
	J. M. Stewart	Research Psychologist	LBEB NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, LBEB, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.1	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As a population of mice reaches optimum density the relations among members of each subgroup are examined with respect to pair bonding, acquisition of social roles and the development of social velocity and dominance hierarchies. In such contexts rodent vocalizations and their temporal variations are examined with particular emphasis on frequency, duration and amplitude. Vocal patterning exhibited by mice in optimum-sized groups will serve as a base line for judging the degradation of capacity for vocal communication that developes with crowding. This project includes part of discontinued projects Z01 MH 00828, 29, and 30.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the principles of group organization, structure, and intragroup communication which operate to foster development of optimal-sized groups with respect to the most favorable development of the individual capacities and to the group's ability to contribute to population survival.

Methods Employed: A mnemonic language for rapid recording of behavior has been developed. Utilizing it, 120 hours of observation was conducted on a mouse population at optimum density. A program of designed lengthening of the intergeneration time to 200 days for mice has been instituted with two to three litters per female removed before a new generation-cohort is permitted to survive. This optimizes the opportunity for improving learned behavior. This strategy improves the conditions for rearing and social organization in each generation. In addition 160 hours of narrative, and 10 surveys of individual characteristics and residence were conducted. At the completion of this phase of data collection, a representative sample of vocalizations was recorded utilizing directional microphones and a PDP-11 computer.

Major Findings: The data base on intra-group social organization and vocal communication of a mouse population has just been completed. No formal analyses have been prepared. However, considering the intimate association with this population during data gathering as it reached optimum density, it is quite apparent that the more effective design of the physical environment and the longer inter-generation time has permitted the development of better integrated individual behavior and intra-group social organization.

Significance to Biomedical Research and the Program of the Institute: All animals, including humans are adapted to develop and live most effectively within small groups. However, urban induced anonymity, frequency of changing residence, and crowding reduce the probability that meaningful interactions will occur at the appropriate intensity and frequency. Our studies of the principles of group organization among rodents contribute to the understandings about requirements of group living.

Proposed Course: By the end of the next year preliminary analyses of vocalizations and group process in an optimum density population of mice should be completed. These studies on vocalization and inter-group process will continue as the population increases in density. In addition, we will have completed the data collection phase on the two mixed strain groups of rats referred to in Z01 MH 0836-01 LBEB.

Publications:

Calhoun, J. B.: "Looking Backward" from the beautiful ones. In W. R. Klemm (Ed.): Discovery Processes in Modern Biology. Huntington, N.Y., Robert E. Krieger Publishing Co., 1977, pp. 26-65.

Burkholder, J. H.: The effects of prenatal maternal crowding on subsequent maternal and social behavior in CD Strain Charles River Rats. Doctoral Dissertation, The University of Akron. June 1977.

Calhoun, J. B.: Crowding and Social Velocity. In: Arieti, S. and Chrzanowski, G. (Eds.): New Dimensions in Psychiatry. New York, John Wiley Sons, 1977, pp. 27-45.

Deitchman, R., Sanders, R. E., Burkholder, J. H., and Newman, I.: Effects of prenatal crowding on rodent maternal and offspring behavior. Psychological Reports. 40: 327-338, 1977.

Slade, N. A. 1976. The Analysis of Social Structure from Multiple Capture Data. J. Mammal. 57: 790-795.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00835-01 LBEB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Crowding and Population Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. A. Slade	Intergovernmental Exchange Fellow	LBEB NIMH
Other:	George G. Ross	Visiting Scientist	LBEB NIMH

COOPERATING UNITS (if any)

none

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.6	0.9

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with phenomena characterizing rodent populations in designed physical habitats as they reach optimum density and as crowding ensues with an increase to eight times this optimum. This year emphasis has been placed on two processes: (1) The increasing proportion of post-reproductive, aged animals accompanying increasing density. An hypothesis that older animals prey on younger ones, in the sense that they prevent the younger ones from fully developing behaviorally, was explored. That is to say, a single specie population can be examined from a predator-prey perspective. Too many predators (i.e., old animals) threatens the population with extinction. (2) Group differentiation within a population to produce a spatial structuring of the relations among groups so that the total pattern of social organization is one exhibiting bilateral symmetry with anterior dominance. This project includes parts of discontinued Z01 MH 00824, and 28.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine those principles of population dynamics that either contribute to adaptation and evolution or by producing pathologies of individual development or of social organization, threaten a population with extinction.

Methods Employed: (1) At each interval of 42 days during a 1600-day history of a mouse population that reached a maximum of 2200 individuals, the number of pre- and post-reproductive individuals was calculated. A theoretical predator-prey model was developed to account for the changing age ratio and the eventual extinction of the population. By iterative computer simulation the best fit between theoretical and observed was determined. (2) Data acquired as described under Z01 MH 00834-01 LBEB provided the basis for evaluating the degree of development of a bilaterally symmetrical social organization in an optimum density mouse population.

Major Findings: With reference to population dynamics: (1) The predator-prey model of the adult to young ratio provided a remarkable fit between observed and theoretical as the number of mice proceeded from below optimum and finally through decline to extinction as the "predator" of old upon young retarded acquisition of behaviors essential for species survival by the young. (2) Although analysis of data has not yet been completed, it is very apparent that the eight groups of mice inhabiting a "universe" of eight radially symmetrical habitat cells have differentiated sufficiently to impose a bilateral symmetry of social organization upon a radially symmetrical physical environment. Two adjoining cells form a "head" or anterior end contain the two most dominant males and the two most stable groups within which young are reared with minimal disturbance and maintenance of litter integrity. At the opposite end, two cells contain the most outcast members of this mouse society. The two mirror-image cells on either side are characterized by inhabitance increasing in stability from "tail" to "head" end of this social organism.

Significance to Biomedical Research and the Program of the Institute: The near future course of human population change (the next 200 years) will likely involve halting further increase and then bringing the population level down toward present levels. To do so will necessitate a rapid shift in age structure. Well before the world population can be brought back to current levels, over half the population will be in the post-reproductive category, and less than 15% in the pre-reproductive age. If the theory of psychological predation on young by the old developed by Drs. Ross and Slade applies to humans, it represents a warning about the possibility of terminating further cultural evolution.

Proposed Course: Natality and mortality will be analyzed from data accumulated on prior studies of mouse populations permitted to proceed to the maximum crowding associated with cessation of reproduction. Intergroup organization and its likely decay will be followed in a contemporaneous mouse population until it reaches four times optimum density.

Publications:

Koeppel, J. W., N. A. Slade, K. Harris, and R. S. Hoffmann. 1977. A Three-Dimensional Home Range Model. J. Mammal. 58: 213-220.

Ross, G. G. and N. A. Slade. A Predator-Prey Viewpoint of a Single Species Population. J. Theor. Biol. In press.

Slade, N. A. 1977. Statistical Detection of Density Dependence from a Series of Sequential Censuses. Ecology. 58:--. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00836-01 LBEB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Conceptual Adaptation and Evolution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	W. M. Baum	Research Psychologist	LBEB NIMH
	J. H. Burkholder	Research Psychologist	LBEB NIMH
	G. G. Ross	Visiting Scientist	LBEB NIMH
	J. M. Stewart	Research Psychologist	LBEB NIMH

COOPERATING UNITS (if any)

none

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A theory is being explored that presupposes development of a network of nodes within the central nervous system to store and interrelate experiences codified as concepts. The theory holds that as individuals develop or species evolve, the complexity and number of concepts (size of nodal network) increases. (1) Wild and domesticated rats are provided the opportunity to enlarge the complexity and number of concepts by introducing conditions which foster cooperation, pair bonding, task oriented, sub-group formation, and communication network development. On the human level two avenues of development are proceeding. (2) A large-scale citation data base is being developed on the impact of the built environment on the health and welfare of people. This domain of research literature will be used as the main base for inquiry into a human CNS concept network, whose computer simulation and use may help human survival and further evolution. (3) A second compact data base has been developed. It consists of 169 1500-word statements written during 1976-77 for the project by leading investigators concerned with adaptation, environment and population. This project includes parts of discontinued 00823,26, 28, 30,31

Project Description:

Objectives: To determine the principles whereby concepts (codifications of experience that influence later behavior) which enhance individual adaptation and survival, and species evolution, are acquired, transmitted, enlarged and integrated into more complex sets.

Methods Employed: (1) Two groups of 24 rats, each consisting of 2 pairs each of 2 strains of wild rats and 4 strains of domesticated rats were established in compartmentalized bilaterally symmetrical environments. Passive resonator implants in each rat permitted recording, identity, time, place, and direction of travel of each rat as it moved through electromagnet fields surrounding doors between compartments. One compartment contained 4 paired drinking situations. At the end of the reporting year the rats had just been transferred from adlib to cooperative drinking condition. (2) Under joint HEW-HUD sponsorship a contract has been let to develop better accessibility to published literature related to the major concerns of the Unit for Research on Behavioral Systems. (3) Leading investigators in the fields of medicine, behavioral sciences, biology, mathematics and physics, and the policy sciences, at our request, have provided us with currently written, specifically for us, 169 condensed 1500-word statements about important, researchable problems relating to adaptation, environment and population. These form a most up-to-date 250,000-word data base about concepts relating to the research objectives of the Unit.

Major Findings: None yet. However, with reference to the third aspect of this project on conceptual evolution, some preliminary conclusions are already apparent. All 169 1500-word statements have been read. Certain major themes which will form chapters in a to-be-published volume are already apparent. These include emphasis on the theme of knowledge and strategy of inquiry, policy formation, language of the environment, crisis resolution, social stress, and principles of evolution. Overall they point to the archaic character of present research bearing on survival, development and evolution of humankind.

Significance to Biomedical Research and the Program of the Institute: The whole evolutionary process may be phrased in terms of increasing abilities to acquire, create, and utilize concepts. Means for realizing these abilities have proceeded from somatic non-brain mechanisms, through brain function, to extra-organismic processes and protheses. The present project is directed toward elucidating principles and procedures for furthering this evolutionary process.

Proposed Course: (1) Acquisition of concepts by members of two groups of rats will be followed as they are successively faced with learning simple, cooperative behavior, intersex bonding behavior, and intra-social-class collaboration. (2) Calhoun will serve as Project Officer to monitor the HEW-HUD feasibility study on increasing access to information on the impact of the built environment on the health and lives of people. (3) The 169 1500-word statements about important, researchable problems related to

adaptation, environment and population will be analyzed to determine how the contained concepts conform to our network theory about relationships among concepts. This analysis will facilitate ordering the statements for most effective publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00881-21 LCM

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Mechanism of Action of Thyroxine and Its Relation to Cerebral Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM	NIMH
OTHER:	G. G. Fitzgerald	Staff Fellow	LCM	NIMH
	E. E. Kaufman	Research Chemist	LCM	NIMH
	T. Nelson	Guest Worker	LCM	NIMH
	G. D. Grave	Guest Worker	LCM	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

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NIMH, ADAMHA,NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is the elucidation of the role of the thyroid hormones in the maturation and development of the brain and the influence of the hormones in the regulation of cerebral metabolic processes. Earlier work done previously in this project demonstrated a stimulating action of thyroid hormones on protein biosynthesis. The mechanism of action of thyroxine remains the primary focus of this project. The effects of thyroid hormones on radioactive amino acid incorporation into protein are examined in cell-free protein synthesizing systems from various mammalian tissues, such as liver, brain, reticulocytes, etc. Studies are occasionally carried out in vivo, in which the uptake of amino acids into protein in various tissues is measured in the intact animal or the effects of experimentally induced thyroid disease on various metabolic activities are examined. A wide variety of enzyme purification and assay procedures are also employed. Enzymes are purified from brain or liver homogenates by detergent treatment, salt fractionation, gel adsorption and filtration, ion exchange chromatography, etc. Assays of enzyme activities utilize chemical analyses, spectrophotometric techniques, and Geiger-Mueller and liquid scintillation radioactivity measurements.

Project Description:

This project is directed at the elucidation of the role of the thyroid hormone in the maturation and development of the brain and the influence of the hormone in the regulation of cerebral metabolic processes. A necessary intermediate objective is the determination of the molecular mechanisms of the physiological and biochemical actions of the thyroid hormone. Previous work in this project demonstrated a stimulating action of thyroid hormones on protein biosynthesis. Although the mechanism of this effect on protein synthesis remains a major area of interest, recent work in this project has shifted more to studies of cerebral metabolic processes which may be influenced by the thyroid hormones and may play important roles in a variety of disease states.

The effects of thyroid hormones on various enzyme activities and on radioactive amino acid incorporation into protein are examined in cell-free systems from various mammalian tissues, such as liver and brain, and also in purified enzyme systems. Studies are occasionally carried out in vivo, in which the uptake of amino acids into protein in various tissues is measured in the intact animal or the effects of experimentally induced thyroid disease on various metabolic activities are examined. A wide variety of enzyme purification and assay procedures are employed. Enzymes are purified from brain or liver homogenates by detergent treatment, salt fractionation, gel adsorption and filtration, ion exchange chromatography, etc. Assays of enzyme activities utilize chemical analyses, spectrophotometric techniques, and Geiger-Mueller and liquid scintillation radioactivity measurements.

A major accomplishment of this project thus far has been the demonstration for the first time that thyroxine stimulates protein biosynthesis and that this effect is probably primary to many of the generally recognized physiological actions of the thyroid hormones. Thyroxine, administered in vivo or in vitro, stimulates amino acid incorporation into protein in cell-free rat liver preparations. Conversely, hypothyroidism induced by surgical thyroidectomy, results in a reduction in the rate of amino acid incorporation into protein in similar preparations, indicating that the thyroid effect on protein biosynthesis is tonically active and physiological.

The biochemical mechanism of the thyroxine stimulation of protein biosynthesis has been studied in considerable detail in in vitro systems. These studies have been carried out over a number of years and have demonstrated that the thyroxine stimulation of protein synthesis is exerted by two mechanisms. There is a direct effect on the translational activity of ribosomes leading to accelerated completion and release of newly-synthesized polypeptide chains followed, in vivo only, by an increased synthesis and accumulation of ribosomes, i.e., a transcriptional effect. The transcriptional effect is not a direct action of the hormone but a cellular response or compensation for some earlier effect. The early effect is the

one most likely to lead to the elucidation of the primary mechanism of action of the thyroid hormones and is the one which has been of greatest interest to the Section.

The early translational effect on protein synthesis, has, however, also been found to be secondary to an even earlier direct, energy-dependent reaction between thyroxine and the mitochondrial components of the cell. A still unidentified product of this reaction which acts on the ribosomes to stimulate their translational activity has been found. Work has been continuing to isolate, purify, and identify this active factor. It is now known to be a small, dialyzable, heat-stable, acid-labile, anionic, organic, probably non-lipid substance. Continued work on it has increased the knowledge of its properties and narrowed the range of possibilities, but its exact identity remains to be established.

This research has already led to the explanation of the change in sensitivity to thyroid hormones which occurs in brain during development. The brain responds to thyroid hormones in a manner similar to other organs during the period of its development and maturation. Once mature, however, it loses its responsivity to the hormones. This change can be seen in the effects of thyroid hormones on oxidative metabolism, protein synthesis, etc. One outcome of this project has been to show that the loss of sensitivity of the brain to thyroxine with development is the result of some change in the mitochondria so that they lose the ability to react with thyroxine and form the active factor responsible for the stimulation of protein synthesis.

Studies have been continued on the nature of the reaction between thyroid hormones and the mitochondria and the changes produced in mitochondria in the course of the reaction. It has long been known that thyroid hormones cause swelling and structural changes in mitochondria. An interesting finding resulting from the present studies has been the fact that *in vitro* thyroid hormones cause a very rapid loss of pyridine nucleotides, particularly NAD^+, from the mitochondria of liver. Mitochondria of mature brain, which also fail to react with thyroid hormones in the preliminary reaction leading to stimulation of protein synthesis, fail to exhibit this hormonal effect on their NAD^+ content. The effect *in vitro* is so rapid that it precedes the effect on mitochondrial swelling and cannot, therefore, be a consequence of the structural changes in these organelles. That the effect, which was first observed with mitochondria *in vitro*, may have physiological significance is suggested by experiments in which a single injection of a very small dose of hormone *in vivo* leads to a significant reduction in the mitochondrial NAD^+ content. The time course of the effect *in vivo* has been studied during the past year. The results indicate that this effect occurs within 8 hours and precedes many of the metabolic effects *in vivo* attributable to thyroid hormones. This effect could have very broad implications. Actions of thyroid hormones are observed on very many metabolic processes in sensitive tissues. NAD^+ is a ubiquitous cofactor for many dehydrogenase reactions which are intimately involved at one point or another in most metabolic pathways. This action of thyroid hormones may, therefore, represent the basis for many subsequent effects of the hormone on a variety of processes.

The thyroid hormones promote the structural, functional, and biochemical maturation of the brain. In addition to the studies of the mechanism of actio of the thyroid hormones, the Section has also been studying the nature of the biochemical changes which occur in the brain with maturation. A prominent developmental change in the brain is a rise in the level of mitochondrial enzymes involved in oxygen consumption and energy metabolism. One of these, D-β-hydroxybutyric dehydrogenase (BDH), was found in this Laboratory to rise with brain development just like other mitochondrial enzymes but, unlike these others, such as cytochrome oxidase and succinoxidase, to decline again gradually following weaning. Evidence has been obtained that the changes in the level of this enzyme in brain are, at least, in part related to nutritiona factors, e.g., the high fat content of maternal milk. Hyperthyroidism shifts the developmental pattern in this enzyme to an earlier age (e.g., it causes a more rapid and earlier rise in the enzyme level in the brain, an earlier achievement of its peak level, and an earlier decline). In the course of these studies an interesting observation was made which might be a valuable model for the elucidation of the mechanism of action of thyroxine in mitochondria. Physiologically active thyroid hormones and their analogues were found in vitro to inhibit BDH activity at extremely low concentrations. The inhibition was found to represent a competitive inhibition by the hormone of the binding of the normal substrates, one of which is NAD^+, to the enzyme. The enzyme is membrane-bound in mitochondria. After considerable effort it has been solubilized and partially purified several-fold from both brain and liver. Efforts are continuing to achieve complete purification. The puri enzyme is to be used to study the mechanisms of the thyroxine-inhibition as well as the mechanisms of the regulation of the enzyme's activity through all its changes during postnatal maturation of the brain. It has already been found with the partially purified enzyme that the enzyme requires a phospholipid cofactor normally present in mitochondria for its activity. This cofactor has been identified as a species of lecithin which can be partially substituted for in vitro by commercial beef heart or egg lecithin. The natural cofactor has been partly purified, and progress has been made to identify the nature of its fatty acid composition. The dioleoyl-lecithin is the most active species examined, but the exact identity is still under investigation.

The Section has recently become interested in the function of this enzyme in the body. D-β-hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D-β-hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from cat catabolism, and it is converted to D-β-hydroxybutyrate by action of the enzyme. Once formed, however, D-β-hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role the enzyme plays. Why does the body not utilize the directly formed acetoacetate rather than convert it first to D-β-hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is

evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility existed that the role of the enzyme is protective to keep the acetoacetate concentrations low by storing it, in a sense, as D-β-hydroxybutyrate. It is noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their prior conversion to acetylCoA. The pathway is the conversion of β-hydroxybutyrate to acetoacetate which then displaces succinate from succinylCoA to form acetoacetylCoA. The acetoacetylCoA is then converted to acetylCoA and enters the Krebs Cycle. For every acetoacetate molecule thus metabolized, there is one succinylCoA degraded. SuccinylCoA is required for the formation of GTP which is the precursor of cyclic GMP. Excessive use of ketone bodies can theoretically, therefore, lead to depletion of available GTP and, therefore, also cyclic GMP. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter, acetylcholine. Its depletion can be expected to result in depression of cholinergic functions in brain and lead possibly to depressed functional activity and even coma. The possibility that ketosis may lead to coma by depletion of brain cyclic GMP is currently being studied in this laboratory. A radioimmune assay for cyclic GMP has been established and preliminary results do indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain.

Some other recent findings in this project have brought it into closer interrelationship with the project on the [^{14}C]deoxyglucose method. It had been found with the [^{14}C]deoxyglucose method that γ-butyrolactone produces a profound trance-like state with widespread depression of cerebral glucose utilization. The γ-butyrolactone is not the active substance; it is first hydrolyzed to γ-hydroxybutyric acid which is the active agent. γ-Hydroxybutyrate has been identified as a normal constituent of brain, but normally present in very small amounts. Studies are in progress to determine whether or not it is elevated in diabetic coma.

At the present time there is very little known about the source and fate of the γ-hydroxybutyrate normally present in brain. There is good evidence that it is oxidized via the Krebs Cycle, but there is no knowledge of its mode of synthesis, and there is nothing at all known of the enzymes involved in its synthesis. Current studies in this laboratory have led to the isolation and partial purification of one enzyme in brain that catalyzes the interconversion of γ-hydroxybutyrate and its oxidation product, succinic semialdehyde. The enzyme is a reversible $NADP^+$ linked oxidoreductase which

could function both in the oxidation of γ-hydroxybutyrate or its formation by reduction of succinic semialdehyde. Succinic semialdehyde is normally formed in brain from the metabolism of GABA, and this enzyme would provide a link between γ-hydroxybutyrate and both the GABA metabolic pathway and the Krebs Cycle. The enzyme has been found to be present not only in brain but in even larger amounts in liver. Studies are continuing to determine the role of th enzyme in the origin and fate of brain γ-hydroxybutyrate and the regulation of its level in brain.

The behavioral effects of exogenously administered γ-hydroxybutyrate are very similar to those seen in hibernation, a phenomenon that is still not understood. The possibility that γ-hydroxybutyrate may be involved in hibernation is being examined in this laboratory. Preliminary studies in the hibernating hamster indicate that there is a significant increase in the level of the enzyme that oxidizes γ-hydroxybutyrate in brain but not in liver. These studies will be continued and will be extended to the measurement of γ-hydroxybutyrate levels in brain in hibernation.

A unique feature of the cerebral metabolism is its apparent lack of response to high circulating levels of thyroid hormone. An understanding of the basis of this unique behavior may reveal information concerning the metabolism of the brain in health and disease. Also, the mechanism of action of thyroxine has been under investigation for many decades, but thus far a satisfactory explanation of the mechanism by which it increases metabolic rate, stimulates metamorphosis and growth, or causes the many disturbances in body physiology and biochemistry in thyroid disease has eluded investigators. The solution of this problem could then lead to fundamental advances not only in the understanding of how this important hormone regulates bodily functions but also of the nature of bodily processes on which it acts. The clarification of the relationship between its mechanism of action and its effects on development of the brain might also lead to a better understanding of the many biochemical processes underlying the maturation of the brain and the basis of the mental retardation which occurs in neonatal hypothyroidism; e.g., cretinism. Studies in this project on ketone body metabolism may also lead to an explanation of the coma which occurs in a number of metabolic diseases characterized by ketosis.

The project will be continued essentially on the course already indicated. The major avenues to be pursued are: (1) the attempts to isolate, purify, and identify the product of the thyroxine-mitochondrial reaction responsible for the stimulation of protein biosynthesis; (2) further studies on the elucidation of the mechanism of the stimulation of the protein biosynthetic processes; (3) further study of the mechanism of the thyroxine effects on mitochondrial NAD^+ content and its consequences; (4) further studies of the biochemical differences between mature and immature brain and the role of thyroxine in the biochemical changes leading to maturation and development of the brain; (5) purification of brain D-β-hydroxybutyric dehydrogenase; (6) studies of the mechanism of the inhibition of D-β-hydroxybutyric dehydrogenase by thyroxine; (7) studies of the mechanisms of the

disturbances in cerebral metabolic processes in coma associated with metabolic diseases and ketosis; (8) isolation and purification of the enzymes involved in the formation and metabolism of γ-hydroxybutyrate; (9) assay of cyclic GMP and γ-hydroxybutyrate in ketosis-associated coma; and (10) studies of the possible role of γ-hydroxybutyrate in hibernation.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00882-10 LCM

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Regional Cerebral Circulation and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	C. Kennedy	Guest Worker	LCM	NIMH
	O. Sakurada	Visiting Associate	LCM	NIMH
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	K. Pettigrew	Mathematical Statistician, Theoretical Statistics & Mathematics Branch	B	NIMH
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9.5	4.5	5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [^{14}C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [^{14}C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. All the theoretical and technical problems have been solved, and the method is now in use for the determination of normal values in the conscious and anesthetized rat. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping functionally, and therefore metabolically, linked structures in the CNS, the method is being used to map the functional visual pathways in the monkey. It is also being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

PHS-6040
(Rev. 10-76)

Project Description:

Previous work in this Laboratory several years ago led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate quantitatively between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the

cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed how to design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.483, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey; its value has been found to be 0.344.

All the theoretical and technical problems have been solved, and the method is now completely operative. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [^{14}C]deoxyglucose is injected, and arterial plasma concentrations of [^{14}C]deoxyglucose and glucose are measured from the time of injection till 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [^{14}C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The method has been applied to normal conscious and anesthetized rats and conscious monkeys. The values obtained cannot be directly compared to any other data in the literature since measurement of local cerebral glucose consumption has never previously been possible, but the average of the local values is very close to the reported values for overall average brain glucose utilization in those two species. Anesthesia has been found to depress cerebral glucose consumption throughout the brain. Carbon dioxide inhalation has been shown to depress local glucose consumption even though it increases cerebral blood flow. Local cerebral blood flow has been found in the normal animal to correlate very closely with local cerebral energy metabolism, a relationship which had been hypothesized but never previously proved. Increased local functional activity produced by electrical stimulation or by experimentally induced focal seizures following local penicillin

injection results in markedly increased local glucose consumption in appropriate regions of the CNS. The studies with experimental focal epilepsy have established the existence of a "mirror focus" contralateral to the epileptogenic focus. These results have established once and for all the often debated but never previously proved fact that energy metabolism in the CNS is closely coupled to functional activity as it is in all other tissues.

Studies have been carried out in the rat on the effects of γ-butyrolactone, a drug alleged to block specifically dopaminergic synapses in the brain. A surprising result was that this agent, which produces a trance-like state, also depresses local glucose consumption throughout the brain. Indeed, the magnitude of the reduction, which is dose-dependent, can be as great as that seen in profound hypothermia, which is used by cardiac and neurosurgeons to reduce energy metabolism in the brain and thus protect it during surgical procedures necessitating interruption of cerebral blood flow. The effects of the drug are completely reversible. Normal consciousness, EEG, and behavior are recovered approximately 4 hours after the injection, and the animal appears normal. This finding may have enormous practical potentialities. It is conceivable that the beneficial effects of hypothermia without its potentially dangerous side-effects and complex technical procedures may be achieved with this drug. These studies will be continued to ascertain whether, in fact, it is safe to try it out in man for surgical procedures.

Studies have been completed on the effects of LSD and of acute morphine administration, morphine addiction, and morphine withdrawal on local cerebral glucose utilization. The morphine studies are being carried out in collaboration with Dr. Werner Klee. These studies have demonstrated selective, dose-dependent depressions of energy metabolism in specific regions of the brain. Morphine addiction has been found to result in significant reductions of glucose utilization in a number of structures of the brain, particularly those of the limbic system. Acute withdrawal from addiction by naloxone administration results in a reversal of this reduction in several of those structures, most prominently in the habenula. An interesting observation is that naloxone alone, when administered to normal rats, reduces glucose utilization in many of the same structures affected by morphine addiction. Naloxone, which is used as a morphine antagonist, has generally been believed to be inactive by itself. These results suggest, however, that it is active, possibly by acting on the receptor sites normally acted upon by the recently discovered endogenous "opiate polypeptides", i.e., the enkephalins.

Studies have been carried out on the effects of amphetamine in the rat. This dopaminergic agonist produces pronounced increases in glucose utilization in selected structures of the brain, in particular the components of the extra-pyramidal dopaminergic motor system, i.e., caudate nucleus zona compacta and zona reticulata of the substantia nigra, etc. A markedly affected structure was the sub-thalamic nucleus, which had previously not been suspected to be dopaminergic, but in follow-up studies based on those of this laboratory, has been found to contain an active dopamine-sensitive adenylcyclase.

Studies on the effects of the β-adrenergic blocking agent, propranolol and the α-adrenergic blocking drug, phentolamine, have led to surprising results. These drugs have directly opposite effects selectively on the auditory system of the rat. Propranolol, which is presently being widely touted as an anti-hallucinogenic agent in schizophrenia and other psychoses, was found to depress markedly the rates of glucose utilization throughout the entire auditory system from the cochlear nucleus to the auditory cortex. In contrast, phentolamine, which has mild depressant effects in most other parts of the brain, has exactly the opposite effects in the components of the auditory system, namely a pronounced increase in glucose utilization.

In the course of the development of the method it became apparent that even without complete quantification, the autoradiographs represented pictorial displays of the relative rates of energy consumption in all parts of the brain. For example, they demonstrated clearly that the synapse-rich areas, e.g., layer 4 of the neuropile in the cerebral cortex, are the most metabolically active areas of the cortex. It also became apparent that inasmuch as local glucose consumption changes in response to changes in functional activity, regions of the brain which were turned on or off functionally and metabolically in altered physiological states could be mapped simultaneously throughout the brain. The efficacy of this technique of mapping by "evoked metabolic response" has been demonstrated in the visual and auditory systems of the rat and monkey. All the regions involved in visual functions have, for example, been shown to alter activity and be displayed on the autoradiographs after occlusion of vision of one eye and stimulation of the other. By this technique the visual cortical locus of the representation of the blind spots of the visual fields has been demonstrated in the monkey and its shape and dimensions defined. The resolution of the method is surprisingly good. It has clearly demonstrated the ocular dominance columns described by Hubel and Wiesel, which are believed to represent the structural and functional organization of the visual cortex responsible for the integration of the two images from the two eyes in animals with binocular vision. Application of the method to the newborn monkey has demonstrated that these columns are already organized at birth. Patching of one eye in the newborn for 3 months leads to a reorganization of the visual cortex so that the portions representing the blocked eye are taken over by the portions of the visual cortex subserving the open eye. Such plasticity is not present in the mature monkey brain.

Studies are in progress to determine the mechanisms by which glucose utilization is coupled to functional activity. One possibility is that increased functional activity increases extracellular K^+, and it is the increased extracellular K^+ that stimulates energy metabolism by activation of the Na^+,K^+-ATPase. Experiments have been carried out in which KCl is applied to the parietal cortex trans-durally by its topical application through a hole in the skull. Such application of the KCl markedly stimulates glucose consumption in the underlying cortex, probably by direct action, while depressing metabolic activity in subcortical structures, probably by reflex or neurogenic reduction of functional activity. This action of KCl is blocked by ouabain which is an inhibitor of the Na^+,K^+-ATPase. These results support the hypothesis that changes in extracellular K^+ mediate the coupling of functional activity and energy metabolism in the brain.

The method has been used to study the acute and delayed effects of hyper-osmolar opening of the blood-brain barrier. The barrier was opened by unilateral carotid arterial injections of hyper-osmolar mannitol. Shortly after the barrier is opened, there is metabolic evidence of punctate regions of seizure-like activity on the side of the barrier opening. The effect is apparently reversible, for those areas of markedly increased metabolic activity return to normal after a period of an hour or more following opening of the barrier.

Several lines of further investigation are planned with the method. One line is methodological by which it is hoped to improve the resolution of the method to the microscopic or single cell level. Another line is to exploit the method at its present state to studies of the effects of aging, psycho-active drugs, metabolic comas, development and maturation of the brain, and hormones such as thyroxine known to have important actions on the maturation of the brain.

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1. Collins, R. C., Kennedy, C., Sokoloff, L., and Plum, F.: Metabolic anatomy of focal motor seizures. Arch. Neurol. 33: 536-542, 1976.

2. Sokoloff, L.: [1-^{14}C]-2-deoxy-d-glucose method for measuring local cerebral glucose utilization. Mathematical analysis and determination of the "lumped" constants. Neurosciences Res. Prog. Bull. 14(4): 466-468, 1976.

3. Reivich, M., and Sokoloff, L.: Application of the 2-deoxy-d-glucose method to the coupling of cerebral metabolism and blood flow. Neurosciences Res. Prog. Bull. 14(4): 474-475, 1976.

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9. Sokoloff, L.: Mapping Brain Activity by Measurement of Local Brain Metabolism. In Rosenfeld, Anne H., and Rosenfeld, Sam A. (Eds.): An NIMH program report. U. S. Department of Health, Education, and Welfare, Public Health Service Publication No.(ADM) 76-353. Washington, D. C., U. S. Government Printing Office, 1976, pp. 1-13.

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15. Sokoloff, L.: Relation between physiological function and energy metabolism in the central nervous system. J. Neurochem., in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00900-21 LCM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on Myelin and Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year we have continued to delineate the region of the guinea pig myelin basic protein that is highly encephalitogenic in Lewis rats. We have found that an 11-residue region, Gln-Lys-Ser-Gln-Arg-Ser-Gln-Asp-Glu-Asn-Pro, is highly active at a level of 0.5 nmole (0.66 μg). Staphylococcal proteinase has been used to cleave specifically the basic protein at aspartyl and glutamyl residues. The resulting peptides are being used to define the immunologically active regions of the protein. A study on the hydrodynamic properties of the basic protein has so far shown that the protein undergoes considerable contraction and reduction in asymmetry at high pH as well as in concentrated NaCl solutions.

Project Description:

The objectives of this study are to continue to examine the relationships between chemical structure and biological activities of the myelin basic protein. The biological activities include the ability to (A) induce delayed hypersensitivity leading to an autoimmune disease, experimental allergic encephalomyelitis (EAE), (B) induce delayed hypersensitivity without necessarily causing EAE, (C) induce the formation of circulating anti-basic protein antibodies without concomitant induction of EAE, and (D) provide structural stability in myelin by its interaction with lipids in the membrane.

The methods used include (1) polyacrylamide gel electrophoresis; (2) amino acid analysis; (3) chromatographic fractionation of proteins and peptides; (4) paper chromatography; (5) high voltage electrophoresis; (6) thin-layer chromatography; (7) specific chemical and enzymic cleavage of myelin basic proteins; and (8) analytical gel filtration.

In continuing our studies on the region of the guinea pig myelin basic protein that is highly encephalitogenic in Lewis rats, we have found that activity is present in the eleven-residue peptide Gln-Lys-Ser-Gln-Arg-Ser-Gln-Asp-Glu-Asn-Pro (residues 72-84). This peptide was obtained by cleavage of active peptide (72-88) at the Pro(84)-Val(85) bond with thermolysin. It is the shortest fragment of the guinea pig protein yet shown to be encephalitogenic in Lewis rats. We are currently examining the possible secondary structure of this peptide by molecular model building. The correctness of the models will be checked by examination of the activities of synthetic peptides identical with peptide (72-84), but with certain amino acid replacements. The peptides will be synthesized by Dr. Janis Young at the Space Sciences Laboratory, University of California, Berkeley. These studies will help us to understand not only what specific molecular structure is necessary for induction of EAE in rats, but also the specific conformation of the basic protein in this region of the molecule.

Dr. Nomura has continued his studies on the applicability of certain proteolytic enzymes to cleave the basic protein at specific sites. The enzyme presently under study is a staphylococcal proteinase, which has been reported to cleave only at the carboxyl peptide bonds of glutamyl residues in ammonium acetate or ammonium bicarbonate buffer and at the carboxyl peptide bonds of both glutamyl and aspartyl bonds in phosphate buffer. Dr. Nomura has found, however, with basic protein as the substrate, that cleavage in ammonium bicarbonate buffer occurs at both residues.

Treatment of the guinea pig basic protein with the staphylococcal protease results in the rapid cleavage of two bonds, Asp(32)-Thr(33) and Asp(37)-Ser(38), followed by cleavage of the Glu(118)-Gly(119) bond. These cleavages result in a mixture of the following: peptides (1-32), (1-37), (33-169) (38-169), (33-118), (38-118), and (119-169). Slow cleavage also occurs at the Asp(132)-Tyr(133) bond yielding peptide (133-169). The sequence Asp-Glu-Asn (residues 81 to 83) is relatively resistant to the enzyme. The guinea pig

peptic peptide (89-169) is readily cleaved by the enzyme, producing mainly peptides (89-118) and (119-169). With longer incubation cleavage also occurs at the Asp(132)-Tyr(133), Asp(144)-Ala(145), and Asp(159)-Ser(160) bonds. Several of these peptides are being used by Dr. Driscoll in his studies on the induction and prevention of EAE and the localization of antibody-binding sites.

The myelin basic protein behaves in solution as a highly flexible, or asymmetric, molecule. The polypeptide chain has very little repeating structure of the α-helical or β-pleated sheet type. It is not clear to what extent the protein is flexible and unfolded and to what extent it is rigid and asymmetric, since most of the physicochemical studies that have been carried out previously have not clearly distinguished between the two alternatives. In order to examine the flexibility of the basic protein I have been carrying out gel filtration studies which measure the variation in hydrodynamic volume (Stokes radius) of the protein with pH or NaCl concentration of the buffer. So far the pH range 3.0 to 10.0 has been examined. As the pH is increased from 3.0 to 5.0 the molecule undergoes a gradual shrinkage. Between 5.0 and 6.0 there is a more abrupt contraction, followed by a plateau between pH 6.0 and 6.5. Between pH 6.5 and 7.5 another abrupt contraction occurs, followed by a slight but steady further contraction between pH 7.5 and 9.5. Above pH 9.5 the molecule begins to undergo a very marked shrinkage. These changes in the hydrodynamic volume of the molecule with pH correlate with the progressive decrease in net positive charge of the molecule as the pH is raised, and the curves relating Stokes radius to pH have the general shape of a protein titration curve. The most abrupt transitions in hydrodynamic volume occur in the pK_a ranges of carboxyl groups (pH 5-6), imidazolyl groups (pH 6.5-7.5), and epsilon amino and phenolic hydroxyl groups (above pH 9.5). The results indicate that there is a considerable degree of flexibility in the molecule over a wide pH range.

Experiments in which the hydrodynamic volume of the basic protein has been studied as a function of NaCl concentration at pH 7.0 indicate that the protein undergoes a marked and steady shrinkage as the NaCl concentration increases from 0.1 M to 2.0 M. Above 1.0 M NaCl the solubility of the protein decreases sharply. The effect of NaCl on decreasing the hydrodynamic volume of the protein is probably the result not only of its charge shielding effect (ionic strength) but, more importantly, on its ability to induce order in the structure of water. The latter capacity would increase hydrophobic interactions both within the protein molecule (shrinkage) and between the molecules (precipitation). It would appear from these studies that under certain conditions the basic protein can exist in a highly compact, nearly globular, conformation. This result, if confirmed by other studies, would be highly significant. In the relatively nonaqueous *in vivo* environment of the myelin membrane the basic protein molecule could well have a compact conformation in which intramolecular hydrophobic interactions predominate, rather than interactions between the peptide backbone and water molecules. It may be possible to approximate the native conformation of the basic protein by placing the protein in an aqueous medium of relatively high NaCl concentration.

Myelin basic protein is under intensive investigation in this and a number of other laboratories to determine its role in the structural integrity of the myelin sheath, where it accounts for 30% of the myelin proteins. One of the major problems concerning the protein is its conformation and the nature of the other myelin constituents with which it interacts in vivo. Intimately related to the structure of the protein is its involvement in an experimental autoimmune disease of the CNS, EAE, where it serves as a potent auto-antigen capable of specifically sensitizing lymphocytes which initiate a delayed-type hypersensitivity reaction in the CNS tissues leading to paralytic lesions. To study the properties of the protein responsible for the induction of the delayed hypersensitivity, both with and without concomitant production of EAE, and those responsible for induction of antibody formation alone, we have examined various areas of the basic protein for the relevant activities. Through these studies, as well as physicochemical studies of the protein, we hope to obtain information relevant to the detailed structure of the protein and to molecular events which occur in the afferent part of immune responses in general, and possibly during the early phases of autoimmunity in humans, specifically in multiple sclerosis.

Publications:

Martenson, R. E.: Myelin basic protein: what does it do? In Kumar, S. (Ed.): Biochemistry of Brain. Oxford, Pergamon Press, in press.

Martenson, R. E., Deibler, G. E. and Kramer, A. J.: Reaction of peptide 89-169 of bovine myelin basic protein with 2-(2-nitrophenylsulfenyl)-3-methyl-3'-bromoindolenine. Biochemistry 16: 216-221, 1977.

Martenson, R. E., Kramer, A. J. and Deibler, G. E.: Microheterogeneity and phosphate content of myelin basic protein from "freeze-blown" guinea pig brains. J. Neurochem. 27: 1529-1531, 1976.

Martenson, R. E., Nomura, K., Levine, S. and Sowinski, R.: Experimental allergic encephalomyelitis in the Lewis rat: further delineation of active sites in guinea pig and bovine myelin basic proteins. J. Immunol. 118: 1280-1285, 1977.

Nomura, K., Martenson, R. E. and Deibler, G. E.: Treatment of an encephalitogenic peptide from guinea pig myelin basic protein with α-protease and thermolysin. Isolation of fragments and determination of cleavage sites. J. Biol. Chem. 252: 1723-1727, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00901-22 LCM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunologic Reactivity of Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
OTHER:	H. D. Webster	Head, Section on Cellular Neuropathology	LNNS	NINCDS
	N. Sternberger	Research Associate	LNNS	NINCDS

COOPERATING UNITS (if any)

Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rabbits have been used to induce specific antisera to several different species of basic protein (BP), including homologous (rabbit) BP. With these antisera we have demonstrated several different specific antigenic regions of the BP molecule. Antisera are being used for collaborative experiments on immunocytochemical localization of BP in neonatal rat CNS and in isolated myelin preparations.

PHS-6040
(Rev. 10-76)

Project Description:

During the past several years we have maintained a small rabbit colony solely for the production of anti-CNS tissue antibodies. Individual rabbits have been immunized with different CNS antigens for the purpose of studying the immunogenicity of basic protein (BP) and its possible relationship to serum factors (other than specific antibody) involved in experimental demyelination. As long as the rabbits remain healthy, blood samples are drawn at biweekly intervals. Sera are labeled and stored at -25°C and careful records kept of immunization schedules, disease reaction, etc. All sera have been characterized by a qualitative test for precipitating antibody. A few have been analyzed for soluble antibody and for evidence of anticerebroside. The results of the past year's research can be summarized as follows:

1) Some of the antisera are being used by Drs. Webster and Sternberger for their studies on the localization of myelin basic protein in tissue sections and isolated myelin fractions. Many unsuccessful attempts have been made by other investigators to determine the precise location of BP in the myelin sheath. Studies on the iodination of myelin proteins in intact spinal cord have suggested an internal location of BP (i.e., in the part of myelin derived from the cytoplasmic surface of the glial cell) but they have not been confirmed by electron microscopy (EM).

Dr. Webster hopes to develop the technology which will permit successful electron micrographs of BP stained with immunocytochemical reagents. Light microscopic pictures clearly indicate successful staining, but do not provide the morphologic detail needed. On the other hand the techniques currently used for EM fixation and mounting interfere with the immunocytochemical staining. The technique is of particular importance in extending our knowledge of the mechanisms of myelin synthesis during neonatal development as well as the mechanisms of myelin damage and repair in the adult.

2) Fragments isolated by Drs. Martenson and Nomura for their study of the structure of the rat encephalitogenic site have been tested with various antisera to define specific antigenic regions in the BP molecule. Previously we found that the fragments 1-36, 37-88, 89-169 all were capable of binding to some antisera. (The specificity of sera varied so we knew they were not cross-reacting sites.) The sera which bind 89-169 usually bind 116-169 and there is very little evidence of a strong binding site between 89 and 115 comparable to the major site in guinea pig antisera. At least two sites occur in fragment 37-88. Some sera which bind fragment 37-88 bind both 43-71 and 61-88. Some sera bind only 43-71 and other sera bind only 61-88. Therefore, we can conclude that there must be a specific site between 43 and 61 and a second site between 71 and 88.

3) Specific antisera have also been used to identify an impurity in bovine basic protein which we suspected might be contributing to some of the immunologic activity ascribed to this species of BP. It has been reported that bovine BP, but not guinea pig BP, induces passive cutaneous anaphylaxis in

guinea pigs--a special type of antibody; bovine BP also induced delayed hypersensitivity which did not correlate with disease onset and severity. These two reactions were atypical of homologous (guinea pig) BP and we attributed the reactivity to a heterologous impurity in the bovine preparation.

The impurity has been identified as the α-chain of bovine hemoglobin. Mrs. Deibler recently completed its isolation and identification. By careful fractionation, she was able to obtain BP essentially free of the contaminant and enough of the contaminant essentially free of BP to permit its identification. A critical step in its identification was the induction of precipitating antibody to purified (unknown) protein.

In addition to studying the location of specific antigenic regions in the basic protein molecule, we have undertaken a study of the rabbit encephalitogenic site. Several years ago, it was reported that the peptide, 43-88, isolated from bovine BP contained this site. Recently another investigator reported encephalitogenic activity (in the rabbit) in two different regions of the molecule, one located near the amino terminal end, the other near the C terminal. We have several well-defined fragments and the capacity of making others, which we plan to use in a re-examination of this question. It appears from our preliminary observations that the major site may not be in the peptide 43-88 as originally reported.

Publications:

Alvord, E. C., Jr., Shaw, C.-M., Hruby, S., Petersen, R., Hsu, P. C. and Kies, M. W.: Neuro-allergic reactions in primates. In Shiraki, H., Yonezawa, T. and Kuroiwa, Y. (Eds.): The Aetiology and Pathogenesis of the Demyelinating Diseases. Tokyo, Japan Science Press, 1976, pp. 203-216.

Baker, P. J., Lint, T. F., Siegel, J., Kies, M. W. and Gewurz, H.: Potentiation of C-56 initiated lysis by leucocyte cationic proteins, myelin basic proteins and lysine-rich histones. Immunology 30: 467-473, 1976.

Kies, M. W.: The relevance of immunologic studies on experimental allergic encephalomyelitis (EAE) to clinical investigations on multiple sclerosis. In Shiraki, H., Yonezawa, T. and Kuroiwa, Y. (Eds.): The Aetiology and Pathogenesis of the Demyelinating Diseases. Tokyo, Japan Science Press, 1976, pp. 265-273.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00902-12 LCM

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. F. Driscoll	Guest Worker	LCM	NIMH
OTHER:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)
National Multiple Sclerosis Society
E. C. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Further studies have been carried out on the immunologic mechanisms involved in the induction and prevention of cell-mediated immunity to myelin basic protein: induction, prevention, suppression and treatment of experimental allergic encephalomyelitis (EAE). Our previous hypothesis that the structural requirements for prevention of EAE are the same as those for induction has been confirmed.

The ability of purified basic protein to prevent the disease induced by whole CNS has also confirmed our hypothesis that BP is the only constituent of CNS tissue which is capable of inducing EAE.

PHS-6040

Project Description:

Experimental animals sensitized with either myelin basic protein (BP) or whole CNS tissue develop an immune response to BP, mediated by a class of lymphocytes referred to as thymus-dependent lymphocytes (T cells). These specific "effector" T cells recognize BP in the nervous tissue of the injec animals and (by an unknown mechanism) damage the tissue which contains the antigen (myelin). This damage is demonstrated histologically by an accumulation of inflammatory cells around small blood vessels in the white matter of the cerebellum and spinal cord. The lesions are accompanied by paralysis of the hind quarters and a general disability often resulting in death.

In order for purified BP or BP in CNS tissue to induce EAE it must be combined with killed mycobacteria in a water-in-oil emulsion. BP in the same emulsion without mycobacteria is nonencephalitogenic and in this form can overcome disease induced by BP plus mycobacteria.

The part of BP which is responsible for its encephalitogenic activity (the encephalitogenic site) is known to include nine amino acid residues, one of which is tryptophan. These nine amino acids correspond to residue numbers 113-121 in the intact protein. Do other parts of the molecule have some immunologic activity which could interfere with cells sensitized specifically to the encephalitogenic site? To answer this question, guinea pigs were pre-immunized with whole BP or its fragments (without mycobacteria) and subsequently sensitized with whole BP (plus mycobacteria). We found that the only peptide of BP which could prevent EAE was one which contained the intact encephalitogenic site (residues 89-169). We have now extended this observation to a smaller encephalitogenic peptide, residues 111-169. This peptide is as encephalitogenic as whole BP and prevents induction of EAE by either BP or peptide (89-169) emulsified with mycobacteria. In contrast, the peptide corresponding to residues 116-169 is completely nonencephalitogenic and nonprotective. The addition of 5 amino acids (residues 111-115) has converted this inactive portion of the BP molecule to one which is capable of sustaining bot reactions. This provides further support for our belief that the encephalitogenic site (residues 113-121) is also the site responsible for disease prevention.

Previously we found that two peptides (1-36 and 1-42), neither of which contains the encephalitogenic site, offered modest protection against EAE. However, because they were encephalitogenic at high levels we suspected that they were contaminated by encephalitogenic fragments. We have now further purified these peptides by recycling chromatography. The more highly purifie peptides (1-36) and (1-42) were found to be nonencephalitogenic and nonpro tive. This further strengthens our conclusion that the peptide sequence whic is encephalitogenic is also necessary for protection.

We have also examined the possibility that CNS tissue contains more tha one encephalitogenic substance. If BP is the only encephalitogen in CNS tissue, it should be capable of preventing disease induced by whole tissue i the manner previously described. In studying this question we found that

CNS-induced disease was much more difficult to overcome than BP-induced disease. However, if large amounts of BP are given at the appropriate time during development of the disease state, BP can overcome CNS-induced disease. We concluded therefore that BP is the only encephalitogen in whole tissue. It appears that the conformation of isolated BP is sufficiently different from its conformation in whole tissue to diminish its effectiveness in preventing CNS tissue-induced disease.

Further studies are needed to obtain more specific information on the nature of the intervention process in both BP and CNS tissue induced diseases. These experiments can be done in vitro and such studies are now being planned.

Publications:

Alvord, E. C., Jr., Hruby, S., Petersen, R. and Kies, M. W.: An analysis of antigenic determinants within myelin membrane encephalitogenic protein. In Shiraki, H., Yonezawa, T. and Kuroiwa, Y. (Eds.): The Aetiology and Pathogenesis of the Demyelinating Diseases. Tokyo, Japan Science Press, 1976, pp. 217-226.

Driscoll, B. F., Kies, M. W. and Alvord, E. C., Jr.: Protection against experimental allergic encephalomyelitis with peptides derived from myelin basic protein: presence of intact encephalitogenic site is essential. J. Immunol. 117: 110-114, 1976.

Seil, F. J., Kies, M. W., Rauch, H. C. and Smith, M. E.: Myelination inhibition factor: its absence in sera from basic protein-sensitized animals. In Shiraki, H., Yonezawa, T. and Kuroiwa, Y. (Eds.): The Aetiology and Pathogenesis of the Demyelinating Diseases. Tokyo, Japan Science Press, 1976, pp. 243-253.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00931-04 LGCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on the Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochemistry	LGCB NIMH
OTHER:	P. K. Chiang	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)
J. P. Bader, Lab. of Tumor Virus Genetics, NCI

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Proteins

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-5/6	5/6	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

S-Adenosylhomocysteine hydrolase (EC 3.3.1.1) from beef liver has been purified and crystallized by methods worked out by our laboratory. This enzyme appears to be homogeneous by polyacrylamide gel electrophoresis. It has a molecular weight of about 240,000 when estimated by gradient gel electrophoresis, and analytical ultracentrifugation. Six types of subunits, with molecular weights ranging between 50,000 to 60,000, were revealed by sodium dodecyl polyacrylamide gel electrophoresis. The amino acid components of this enzyme have been analyzed, and there is one glucosamine per enzyme molecule. The kinetic properties of this enzyme are currently being studied, especially with respect to chemical analogs that may substitute for the natural substrates, or act as inhibitors. The chemotherapeutic efficacies of these chemical analogs are being tested in animals and cell culture systems.

PHS-6040
(Rev. 10-76)

Project Description:

The enzymatic transfer of the methyl group of S-adenosyl-L-methionine (AdoMet) yields S-adenosyl-L-homocysteine (AdoHcy) as one of the products of the reaction. In eukaryotes, as far as it is known, the principal pathway for the metabolism of AdoHcy in its hydrolysis to L-homocysteine (Hcy) and adenosine by the action of an enzyme, AdoHcy hydrolase, first described in rat liver by de la Haba and Cantoni. The reaction catalyzed by AdoHcy hydrolase is described in Eq. 1

$$\text{S-Adenosyl-L-homocysteine} \overset{\overset{E}{\rightleftharpoons}}{H_2O} \text{Adenosine + L-Homocysteine}$$

AdoHcy has been found to inhibit powerfully most of the methyltransferases which utilize AdoMet as the methyl donor, but with Ki's that vary over a wide range. Because of different sensitivity of various methyltransferases toward AdoHcy, the regulation of AdoHcy hydrolase by various effectors can be of physiological importance in controlling biological methylations. Some of the important methylated compounds that may be affected are neurohormones or neurohormone precursors (methylated catecholamines, methylhistamines, methylated indolamines and choline), proteins (myosin, histones, encephalitogenic proteins), and nucleic acids (tRNA, mRNA, DNA). The observation that the level of AdoHcy hydrolase in tissues is influenced by nutritional and hormonal factors suggests a regulatory role for this enzyme. Finally, AdoHcy, by virtue of its ability to inhibit betaine-homocysteine methyltransferase, and its ability to activate cystathionine synthase, may play a role in liver in determining whether homocysteine will be remethylated to methionine or utilized for the synthesis of cystathionine.

AdoHcy hydrolase has been characterized only partially in the past. Further work on the enzyme, its tissue and intracellular distribution, its regulation and the effect of analogs and/or inhibitors will be of great physiological interest.

Amino acid analysis of the purified AdoHcy hydrolase revealed the presence of all the common amino acids. Of particular interest is the presence of glucosamine, 1.2 residues per mole of enzyme; thus indicating that AdoHcy hydrolase is probably a glycoprotein.

A total of 43 structural analogs of AdoHcy with modifications in the purine, sugar, 5'-thioether linkage and/or amino acid portion were tested as substrates and/or inhibitors of Ado Hcy hydrolase. It was observed that S-3-deazaadenosyl-L-homocysteine was the only analog that could serve as a substrate of the enzyme as well as AdoHcy itself. When tested in the direction of AdoHcy hydrolysis

coupled with calf intestinal adenosine deaminase, the following were found to be the more potent inhibitory analogs: 5'-deoxy-5'-butylthioadenosine, NY-adenosyl-α,γ-diaminobutyric acid, 9-(5'-deoxy-5'-methylthio-α-D-arabinofurnaosyl)adenine, 5'-deoxy-5'-aminoethylthio-2-chloroadenosine, 3-deazaadenosine and S-3-deazaadenosyl-L-homocysteine. 3-Deazaadenosine is the most potent inhibitor of AdoHcy hydrolase and as well as being resistant to adenosine deaminase. When tested in vitro on isolated rat hepatocytes, 3-deazaadenosine caused a drastic increase in the levels of AdoHcy and AdoMet, with the formation of S-3-deazaadenosyl-L-homocysteine. 3-Deazaadenosine is thus an interesting analog with biological potential. 3-Deaza-adenosine is also effective in preventing virally induced oncogenic transformation of chick embryo cells in cell culture and in decreasing virus formation by infected cells. Moreover, 3-deazaadenosine is able to reverse virus induced malignant transformation of chick embryo cells and to restore normal growth inhibition patterns. The mechanism of this interesting effect is under investigation.

Publications:

Chiang, P. K., Richards, H. H. and Cantoni, G. L.: S-Adenosyl-L-homocysteine hydrolase. Analogs of S-adenosyl-L-homocysteine as potential inhibitors. Molec. Pharmacol., in press.

Chiang, P. K., Cantoni, G. L., Ray, D. A. and Bader, J. P.: Reduced levels of adenosine deaminase in chick embryo fibroblasts by Rous sarcoma virus. Biochem. Biophys. Res. Commun., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00932-05 LGCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Muscle Differentiation in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochemistry	LGCB NIMH
OTHER:	B. W. Uhlendorf	Res. Microbiologist	LGCB NIMH
	S. Scarpa	Visiting Associate	LGCB NIMH
	J. K. Chen	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)
R. S. Adelstein, Chief, Sect. Molecular Cardiology, NHLI

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Proteins

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4-1/3	2-1/3	2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myogenesis in vitro has been much studied as a model of cellular differentiation. The formation of multinucleated non-proliferating, electrically active and contractile myotubes by fusion of undifferentiated, proliferating myoblasts is accompanied by the appearance of muscle specific proteins, such as creatine kinase and myosin, and by the arrest of DNA synthesis. Thus, morphological and physiological changes find a counterpart in a series of biochemical events even though the mechanism(s) responsible for these changes in gene expression is (are) completely unknown.

Project Description:

Myogenesis *in vitro* can be studied using as experimental object L_5, a cloned, permanent cell-line derived from rat skeletal muscle. By appropriate manipulation of the cultural conditions, L_5 myoblasts can be programmed to differentiate into myotubes in a completely reproducible manner over a period of 7-8 days.

The formation of multinucleated myotubes must involve participation of cellular membranes both in cell to cell recognition and in the complex series of events that underly fusion of dozens of mononucleated myoblasts to form multinucleated myofibers.

The interaction of plant lectins with cellular membranes have been the object of extensive investigations in normal, neoplastic and differentiating systems. Concanavalin A, wheat germ agglutinin and other lectins can, by unknown mechanisms, stimulate mitosis and it was of interest to examine the effect of mitogenic lectins on the phenomenon of myogenesis, that, as noted above, is characterized by an arrest of cellular proliferation.

It has been found that Concanavalin A at 3-5γ/ml is capable of inhibiting myogenesis without having any appreciable mitogenic effect. Moreover, Concanavalin A when applied between day 1 and 4 inhibit myogenesis (measured at day 7) whereas it is without effect if applied between day 4 and 7. The arrest of DNA synthesis that is an early manifestation of muscle differentiation is not prevented by Concanavalin A.

The effects of Concanavalin A are mediated through specific carbohydrate determinants on the cell surface. The nature of these determinants have been investigated by means of other lectins of known specifics and by the competitive effect of monosaccharides.

The results of these experiments led to the formulation of a working hypothesis that is consistent with the model of the cell's life proposed by Smith and Martin. An exogenous stimulus (the nature of which is not known) is required to trigger differentiation and to recruit cells to advance to the differentiated state (D_n) through a series of steps D_1, D_2, D_3 etc. Arrest of DNA synthesis is the earliest stage so far characterized (D_1). Concanavalin A acts by inhibiting one of the later steps in the progress from D_1 to D_n.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00933-03 LGCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Study of the S-Adenosylmethionine Synthetase of Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochemistry	LGCB NIMH
OTHER:	P. K. Chiang	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Proteins

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/6	5/6	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purification and characterization of S-adenosylmethionine (AdoMet) synthetase (EC 2.5.1.6) from yeast is currently in progress. We have been able to demonstrate the existence of two molecular species of AdoMet synthetase which exhibit differences in chromatographic behavior and electrophoretic mobility. These two species of AdoMet synthetase have identical molecular weight, approximately 110,000 when estimated by Sephadex G-150. Both forms of the enzyme catalyze the cleavage of inorganic tripolyphosphate. AdoMet synthetase is totally dependent upon added AdoMet for its tripolyphosphatase activity, whereas the tripolyphosphatase activity of AdoMet synthetase I is active without addition of exogenous AdoMet although in its presence, its activity is significantly higher.

PHS-6040
(Rev. 10-76)

Project Description:

S-Adenosylmethionine (AdoMet) synthetase (ATP: L-methionine S-adenosyltransferase) (EC 2.5.1.6) catalyzes the adenosylation of methionine at the expense of ATP. The reaction catalyzed by this enzyme is described by equation 1:

$$\text{Methionine} + \text{ATP} \xrightarrow[K^+]{Mg^{++}} \text{AMe} + \text{PPI} + \text{Pi}$$

The reaction is unusual in several respects. In the first place, it represents the only example of the utilization of the energy of the phosphate bond of ATP for the generation of an energy-rich sulfonium compound. Next, the three phosphates of ATP are cleaved in an oriented non-random fashion to generate pyrophosphate and inorganic phosphate by a tripolyphosphatase that is associated with AdoMet synthetase of yeast. This activity is stimulated by low concentrations of AdoMet, perhaps by an allosteric activation.

In the course of the purification of AdoMet synthetase, two forms of this enzyme could be demonstrated. These two forms, tentatively called AdoMet synthetase I and II, can be separated by DEAE-cellulose column chromatography, and exhibit different electrophoretic mobilities when purified. The molecular weight for both forms of AdoMet synthetase has been estimated to be 110,000 by Sephadex G-150. When subjected to SDS polyacrylamide gel electrophoresis both forms of AdoMet synthetase display two polypeptide subunits having molecular weights of 55,000 and 60,000, respectively. Kinetically, both forms of AdoMet synthetase exhibit negative cooperativity with respect to L-methionine and ATP as variable substrates, whereas positive cooperativity was observed in the presence of L-ethionine. In addition to their chromatographic and electrophoretic properties, AdoMet synthetase I and II differ with respect to the properties of their tripolyphosphatase activity, which is an intrinsic component of AdoMet synthetase. The tripolyphosphatase activity of AdoMet synthetase II is completely dependent upon the addition of exogenous AdoMet; whereas the tripolyphosphatase of AdoMet synthetase I is active without addition of AdoMet although in its presence its activity is significantly higher.

The physiological significance of these findings is under investigation. It should be pointed out that mutants have been isolated from E. coli and yeast that have in vivo and in vitro very low levels of AdoMet synthetase and that in these mutants the control of methionine biosynthesis is dramatically altered.

Also variations in the level of AdoMet synthetase have been reported in a few children with hypermethioninemia, in patients with leukemia and in acute schizophrenia. The relationship of these findings with the discovery of two forms of AdoMet synthetase will be investigated.

Publications:

Chiang, P. K. and Cantoni, G. L.: Activation of methionine for transmethylation. Purification of the S-adenosylmethionine synthetase of baker's yeast and its separation into two forms. J. Biol. Chem. 252: 4506-4513, 1977.

Cantoni, G. L.: Formyl metabolism. Encyclopedia of Chemistry, V. Florence, Italy, USES Publishers, 1976, pp. 527-532.

Cantoni, G. L.: Methyl group metabolism. Encyclopedia of Chemistry. Florence, Italy, USES Publishers, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00934-05 LGCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on the Biochemical Basis of Narcotic Drug Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. E. Jacobson	Research Chemist	LC NIAMDD
	K. C. Rice	Research Chemist	LC NIAMDD
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COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Proteins

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cells of neural origin (NG108-15) which carry opiate receptors and can be maintained indefinitely in culture are used to study the mechanism of action of morphine and morphine-like peptides (enkephalin, endorphin). These materials exert a receptor mediated dual regulation of adenylate cyclase; a rapid inhibition of activity upon which is superimposed a subsequent increase in the amount of total expressible enzyme activity. This, homeostatic, mechanism may account for tolerance to and dependence upon the opiates. The enkephalins were found to be much more potent than their presumed precursors, the endorphins as mediators of dual regulation. Some actions of morphine are neither stereospecific nor mimiced by opioid peptides. These actions appear to be mediated by a different class of receptor which may play a role in dependence.

PHS-6040
(Rev. 10-76)

Project Description:

Our studies of the mechanism of morphine action have continued to make rapid progress, largely because of the favorable properties of the neuroblastoma x glioma cell line NG108-15. This hybrid cell has a large number of opiate receptors on its surface which are functionally coupled to the enzyme, adenylate cyclase, as inhibitors, as we had demonstrated earlier. These cells become addicted to morphine and other opiates as a result of being cultured in their presence for 20 hours or longer. The addictive process is a homeostatic mechanism which serves to maintain cellular cyclic AMP levels near their normal values. The acute effects of opiates are to inhibit adenylate cyclase and thereby rapidly lower cAMP levels; the chronic adaptation is due to a gradual expansion in the amount of enzyme activity which serves to maintain normal cAMP levels even in the continued presence of morphine. Thus, adenylate cyclase is subject to dual regulation by receptor-opiate complexes, a rapid inhibition of enzyme activity upon which is superimposed a subsequent increase in the total amount of expressible activity. The increased enzyme activity is due, at least in part, to changes in the regulatory properties of the pre-existing enzyme rather than to an increase in the number of enzyme molecules in the cell.

Peptides with morphine-like activity have recently been characterized by Hughes and others after purification from brain extracts. These peptides, the enkephalins and endorphins, all seem to be fragments of the pro-hormone ß-lipotropin and can thus be considered neurohormones. We have tested many peptides of this class with adenylate cyclase prepared from NG108-15 cells and find them to be among the most potent, receptor mediated, inhibitors known for this enzyme. Furthermore, when cultures of NG108-15 cells are exposed to large amounts of these peptides for a number of days, they become addicted in exactly the manner found for morphine and the other opiates. Our data, to date, show that the only difference in action between the enkephalins and the classical opiates is a much shorter duration of action of the peptides due, perhaps, to proteolytic degradation. The hybrid cells, which are richly endowed with opiate receptors, are relatively deficient in enzymes which degrade opioid peptides. It has therefore been possible to study the effects of these peptides, subsequent to receptor binding, without serious complications due to their destruction by the adventitious peptidases which are found in great abundance in brain. We have found that, contrary to results obtained with brain *in vivo* and *in vitro*, the pentapeptide methionine enkephalin is a more potent opioid (as measured by the receptor mediated inhibition of adenylate cyclase) than are any of the larger peptide fragments of ß-lipotropin including some, such as ß-endorphin (ß-LPH 61-91), which in

in vivo seem much more potent than is enkephalin. Since enkephalin is very rapidly inactivated in brain, these experiments raise the possibility that enkephalin may, in fact, be the physiologically most important opioid peptide and that the larger peptides may represent a more stable, depot, form from which enkephalin is released upon demand.

Some effects of morphine, in vivo are not mediated by the usual opiate receptors. The hyperresponsivity to audio-visual stimuli seen after microinjection of morphine into the periaqueductal gray or the midbrain reticular formation was found to be elicited by the (+) (unnatural) as well as the (-) (natural) enantiomers of morphine. Analgesia, on the other hand, is observed only upon administration of (-) morphine. These results suggest that the hyperexcitability seen in the morphine abstinance syndrome may, in part, be the result of residual stimulation of this presumed new class of receptor. Since the opioid peptides were found not to cause such excitability, it is conceivable that withdrawal following dependence upon opioid peptides may be much milder than is the morphine withdrawal syndrome.

Our studies of the characterization of the interaction of synthetic opiates with rat brain receptors and as analgesics or antagonists have led to some insights into the forces involved in receptor opiate interactions. Efforts are also currently being directed towards the development of long acting antagonists which may be therapeutically useful and of affinity probes for the covalent labelling of opiate receptors.

Publications:

Klee, W. A., Lampert, A. and Nirenberg, M.: Dual regulation of adenylate cyclase by endogenous opiate peptides. In Kosterlitz, H. W. (Ed.): Opiates and Endogenous Opioid Peptides. Amsterdam, Elsevier, 1976, pp. 153-160.

Lampert, A., Nirenberg, M. and Klee, W. A.: Tolerance and dependence evoked by an endogenous opiate peptide. Proc. Natl. Acad. Sci. U.S.A. 73: 3165-3167, 1976.

Klee, W. A. and Nirenberg, M.: The mode of action of endogenous opiate peptides. Nature 263: 609-612, 1976.

Goldstein, A., Cox, B. M., Klee, W. A. and Nirenberg, M.: Endorphin from pituitary inhibits cyclic AMP formation in homogenates of neuroblastoma x glioma hybrid cells. Nature 265: 362-363, 1977.

Iorio, M. A. and Klee, W. A.: Relationships between opiate receptor binding and analgetic properties of prodine-type compounds. J. Med. Chem. 20: 309-310, 1977.

Klee, W. A.: Opiates and cyclic AMP. In Blum, K. (Ed.): Alcohol and Opiates. New York, Academic Press, 1976, pp. 299-308.

Klee, W. A.: Endogenous opiate peptides. In Gainer, H. (Ed.): Peptides in Neurobiology. New York, Plenum Press, 1977, pp. 375-396.

Jacobson, A. E., Klee, W. A. and Dunn, W. J. III: A quantitative relationship between antinociceptive activity, opiate receptor affinity and lipophilicity. Eur. J. Med. Chem. 12: 49-52, 1977.

Agarwal, N. S., Hruby, V. J., Katz, R., Klee, W. and Nirenberg, M.: Synthesis of leucine enkephalin derivatives: structure-function studies. Biochem. Biophys. Res. Commun. 76: 129-135, 1977.

Rice, K. C., Shiotani, S., Creveling, C. R., Jacobson, A. E. and Klee, W. A.: N-(2,4,5-Trihydroxyphenethyl)normetazocine, a potential irreversible inhibitor of the narcotic receptor. J. Med. Chem. 20: 673-675, 1977.

Sharma, S. K., Klee, W. A. and Nirenberg, M.: Opiate dependent modulation of adenylate cyclase. Proc. Natl. Acad. Sci. U.S.A., in press.

Jacquet, Y. F., Klee, W. A., Rice, K. C., Iijima, I. and Minamikawa, J.: Stereospecific and non-stereospecific effects of (+) and (-) morphine: evidence for a new class of receptors? Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00935-10 LGCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Effect of Small Viruses and Their Nucleic Acids on the Biochemistry of Living Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. K. Das	Visiting Fellow	LMB NCI
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SECTION
Section on Proteins

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prokaryotic gene expression in mammalian cells involving the purine phosphoribosyltransferase enzymes were investigated. Micro assays were developed for these enzymes and a bacterial virus carrying the gene for one of the enzymes was used. The number of clones isolated following viral infection was 100-fold greater than those observed in controls. However, electrophoretic analysis of the enzyme produced in these cells was not diagnostic of a bacterial enzyme. Immunological methods are now being developed. The work on galactosemic human cells has been impeded by the lack of good selection systems to isolate cells expressing the galactose genes. Galactose enzyme assays and nucleic acid hybridization techniques were used to demonstrate the discoordinate gene expression of the E. coli galactose operon after induction of a lambda prophage. These studies have led to a realization that controlled gene expression in heterologous systems will require a detailed knowledge of translational and transcriptional control mechanisms.

Project Description:

Bacterial virus gene expression has been demonstrated in human cells lacking the UDP-gal transferase enzyme (galactosemic fibroblasts) and cells lacking the ß-galactosidase enzyme (GM,-gangliosidosis fibroblasts). In both systems, enzymatic studies have indicated that the enzyme appearing after infection with an appropriate bacterial virus is similar to the bacterial enzyme by physicochemical analysis and, in the case of the ß-galactosidase enzyme, by immunological methods. Further analysis of these systems has been impeded by the lack of selection systems for cells expressing the prokaryotic genes. Strong selection systems have been developed for mammalian cells deficient in the purine phosphoribosyltransferase enzymes. In order to utilize these systems to investigate bacterial virus gene expression in eukaryotic cells, a bacterial virus containing the gene for guanine (and xanthine) phosphoribosyltransferase activity has been grown and purified in large quantities. Also, micro enzyme assays. These assays use ^{14}C labeled substrates and thin layer chromatography to separate the reagents from the product.

Attempts to test bacterial virus effects in whole animal (mice) was thwarted by the death of the animals following injection with some preparations of the bacterial viruses. By the use of the Limulus Assay method it appears that these deaths were due to endotoxin contamination of the virus preparations. Such contamination can be reduced considerably by extracting the virus preparations with chloroform prior to purification by banding in CsCl gradients. Experiments are currently being conducted to test for an increased survival following infection with a virus carrying the bacterial galactose operon in mice on a lethal high galactose diet.

Methods of monitoring blood and galactose levels and the galactose enzymes in the livers of animals in these studies have been developed.

The galactose assays developed in the above studies, coupled with nucleic acid hybridization methods have led to the observation of discoordinate expression of the galactose enzyme in the E. coli galactose operon when the operon is transcribed from a lambda prophage promotor. This finding is in contrast to the normal coordinate expression of the three cistrons of the E. coli gal operon.

The purine phosphoribosyltransferase enzyme assays developed above have permitted a screening study of patients with self mutilating

syndromes in a search for either variants of the Lesch-Nyhan syndrome or defects in other purine pathways which might result in aberrant behavorial problems.

Publications:

Merril, C. R., Gottesman, M., Court, D. and Adhya, S.: Discoordinate expression of the E. coli gal operon after prophage lambda induction. J. Mol. Biol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00936-14 LGCB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Homocystinuria: Methionine Metabolism in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-1/12	2/12	11/12

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obligate heterozygotes for homocystinuria due to cystathionine synthase deficiency have been shown to have elevated plasma concentrations of homocysteine-cysteine mixed disulfide after a standarized methionine load. A case-control study reported that after methionine loads 7 of 25 young patients with angiographic evidence of major coronary disease, but only 1 of 22 controls, had homocysteine-cysteine disulfide concentrations as high as those found in obligate heterozygotes after similar loading. Together with the well-known tendency of homozygotes for cystathionine synthase deficiency to have thrombi and emboli, these findings suggest that heterozygotes for this condition may be at significantly increased risk for coronary artery disease. A questionaire survey to evaluate this possibility has been initiated and is now in the pilot-testing stage.

PHS-6040
(Rev. 10-76)

Project Description:

The objectives of this project are to obtain a detailed knowledge of the biochemistry of sulfur containing compounds in the mammalian body. Inextricably involved also is the biochemistry of transmethylation. Special attention will be focussed upon the regulatory devices which control these areas. We are concerned, too, with the inborn errors of metabolism due to abnormalities in these areas. Definition of the underlying lesions and their pathophysiology may help in the management of these diseases and offers unique help in understanding normal metabolism. As noted in previous annual reports, work on this project has necessarily been severely curtailed because of lack of available professional personnel.

In the past year, the major effort has been devoted toward initiation of a project aimed at evaluation of the possible adverse health effects of the heterozygous condition for homocystinuria due to cystathionine synthase deficiency. This study was suggested by several observations: (a) obligate heterozygotes for cystathionine synthase deficiency can be detected by the presence of an increased concentration of homocysteine-cysteine mixed disulfide in the plasma at specified intervals following a standard oral load of methionine superimposed upon a defined diet. (b) a case-control study found that after methionine loads 7 of 25 young patients with angiographic evidence of major coronary disease, but only 1 of 22 controls, had homocysteine-cysteine disulfide concentrations as high as those found in obligate heterozygotes after similar loading. Slightly lower concentrations of this disulfi were found in 8 patients and 3 controls. Together with the well-established fact that homozygotes for cystathionine synthase deficiency have thrombi and emboli in many vessels leading to stroke and coronary conclusion, these findings suggest that heterozygotes for cystathionine synthase deficiency may be at increased risk for early coronary artery disease. The genetics of this condition are such that one person in 100-200 of the population is probably such a heterozygote. Thus, the public health implications are amplified far beyond those based on the relatively infrequent occurrence of homozygotes. Indeed, a confirmation of a causal relationship between heterozygous cystathionine synthase deficiency and coronary artery disease could account for a substantial portion of the "unexplained" coronary artery disease in the U.S.

To explore this possibility, in collaboration with Drs. Manning Feinleib and Richard Havlik of the Epidemiology Branch of the National Heart, Lung and Blood Institute, and Dr. Harvey Levy, Mass. Gereral Hospital, we are planning a questionaire search of the health history of obligate heterozygotes for cystathionine synthase deficiency. The consulting physician who has diagnosed or reported a case of the disease will be asked to provide the parent with a standardized short form listing disease status of parents as well as grandparents. Simple comparisons of the collated data will then be made between the disease experience in the parents and the expected experience, as derived from the Framingham study, or other population based studies. Further groups consisting of the parents of children with other genetic diseases (e.g. cystinosis, acromegalic dwarfism) will be similarly surveyed to provide additional controls.

Detailed preparations for this study are now finished. Adequate care has been taken to safeguard the participants in this study, and necessary clearances obtained from Human Research Committees, etc. A pilot survey to test the questionaire form is now underway. It is anticipated that this survey can be completed in the next year and that the methodology developed may be useful not only for this particular project, but also in opening the way to evaluation of the public health implications of a number of further genetic diseases about which analogous questions may be raised.

During the past year, some progress has been made in the project to develop mouse models for human genetic diseases. During the last few months of the second contract to study methods for frozen storage of mouse epididymal sperm, Dr. Rapatz was able to obtain many pregnancies with frozen-thawed sperm. In the best medium so far developed, 9 of 84 animals inseminated with frozen sperm (11%) gave birth to a total of 61 young (average of 6.8 per litter). Of 102 animals inseminated with the same, but unfrozen sperm, 23 (23%) gave birth to 178 young (average of 7.7 per litter). These results demonstrate conclusively it is possible to freeze and thaw mouse sperm with retention of fertilizing capacity. More work would be required to obtain better fertilization rates, both in controls and in animals inseminated with frozen sperm. Discussions are now underway to decide whether to pursue this approach or to change to the use of frozen embryos.

Publication:

Uhlemann, E. R., TenPas, J. H., Lucky, A. W., Schulman, J. D., Mudd, S. H. and Shulman, N. R.: Platelet survival and morphology in homocystinuria due to cystathionine synthase deficiency. New. Engl. J. Med. 295: 1283-1286, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00937-12 LGCB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transsulfuration in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. H. Datko	Botanist	LGCB NIMH
	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH
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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3-1/12	3	1/12

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous studies have shown that the alga *Chlorella* synthesizes homocysteine predominantly by transsulfuration, and no activity of direct sulfhydration could be detected. These studies have been extended to the higher plant *Lemna*, which has been shown to synthesize homocysteine also predominantly by transsulfuration. However, in the case of *Lemna* it was convincingly demonstrated that direct sulfhydration did contribute a small proportion (in the range of 5-10%) of the total homocysteine biosynthesized. *Lemna* has been selected for long-term studies on the mechanism of control of methionine biosynthesis in the two pathways. Conditions for optimal growth of *Lemna*, and the limits within which these conditions must be controlled, have been determined. *Lemna* was found to exert a remarkable control over sulfur metabolism which permits both the pool sizes of sulfur amino acid intermediates and the synthesis of organic sulfur compounds to remain essentially unchanged when the plant is presented with a 3000-fold variation in the environmental concentration of inorganic sulfate. Plants growing in the presence of methionine can increase the size of their soluble methionine pool over 50-fold, where it now represents a significant fraction of the total cellular methionine.

Project Description:

Previous work has shown that plants are potentially capable of synthesizing homocysteine (and methionine) by two main pathways - the direct sulfhydration pathway and the transsulfuration pathway. The long-term objective of this project is to determine the relative physiological significance and the mechanism of control of these two pathways.

In the last report we described experiments on the assimilation of sulfate-^{35}S by the alga *Chlorella* (a single-celled, lower plant) growing under steady state conditions with limiting sulfate, that demonstrated that homocysteine biosynthesis *in vivo* proceeded predominantly, if not exclusively, by transsulfuration. No activity of the direct sulfhydration pathway could be detected and, if functional, this pathway, contributed no more than approximately 3% of the total homocysteine biosynthesized. Recent experiments with the higher plant *Lemna* ("duckweed") growing under conditions similar to these described for *Chlorella* have shown that this plant synthesized homocysteine also predominantly by transsulfuration. However in the case of *Lemna* it was convincingly demonstrated that direct sulfhydration did contribute a small proportion (in the range of 5-10%) of the total homocysteine biosynthesized. Our studies with *Lemna* also confirmed an interesting finding previously observed with *Chlorella*: only a small proportion (in the order of less than 1%) of the total cellular soluble cysteine is on the pathway of sulfate assimilation. This finding indicates a very efficient coupling between the synthesis of soluble cysteine and its further metabolism to cystathionine, GSH and protein cysteine.

The plant *Lemna* has been selected for detailed studies on the mechanism of control of methionine biosynthesis in the two pathways. *Lemna* has many advantages which appear to facilitate such studies: ability to grow very rapidly on defined media either photoautotrophically or heterotrophically; ready attainment and maintenance of axenic conditions; continued asexual reproduction with consequent genetic stability; small size which allows randomization of plant samples for stages of life history; natural capacity to take up nutrients from the medium (which facilitates nutritional and radioactive tracer experiments); and (in *L. perpusilla*) well established conditions for changing from asexual to sexual reproduction to permit eventual genetic experiments. These studies, which are still in as early stage, may be summarized as follows:

(1) Despite the popularity of *Lemna* as an experimental material, there is a surprising lack of reports of systematic investigations on the effects of variations in physical and chemical factors upon the growth of this plant. Thus one could be sure about neither optimal conditions for growth, nor the limits within which such variables must be controlled to avoid inadvertant effects on growth. We have defined conditions for optimal growth and the "plateaus" for such factors as temperature, pH, CO_2 concentration, light and macronutrient and micronutrient composition and concentration. These experiments were performed not only under photoautotrophic conditions, but also under heterotrophic conditions to facilitate ^{14}C labeling experiments with a fixed

rather than a gaseous, source of ^{14}C. This phase of our work is almost complete and will provide a standardized set of conditions for our long-term studies on *Lemna*.

(2) Pool sizes of the main sulfur components have been determined in *Lemna* growing under steady state conditions in the presence of increasing concentrations of inorganic sulfate (0.32 μM, 4.5 μM, 14.3 μM, and 1000 μM). Steady state growth at the lower concentrations of sulfate was made possible by use of the "lemnastat," which was described in the last report. New methods were developed which enabled S-methylcysteine and the sulfonium compounds S-methylmethionine and S-adenosylmethionine to be assayed with greatly improved sensitivity. For example S-adenosylmethionine, which could play an important role in the regulation of methionine synthesis in plants, can now be assayed in amounts as low as 0.2 nmoles. Essentially similar growth rates were observed at each of the sulfate concentrations listed. The pool sizes of inorganic sulfate (expressed on a plant basis) increased progressively over 40-fold as inorganic sulfate in the medium was increased from 0.32 μM to 1000 μM. By contrast, the pool sizes of most of the sulfur amino acids assayed (glutathione, homocysteine, soluble cysteine, soluble methionine, S-adenosylhomocysteine, cystathionine, protein cysteine and protein methionine) were essentially unchanged. The pool sizes of S-methylmethionine sulfonium increased progressively by a factor of approximately 5 between 0.32 μM and 1000 μM inorganic sulfate. The pool sizes of S-adenosylmethionine were determined only for plants grown in 14.3 μM and 1000 μM inorganic sulfate, and were essentially identical. These results demonstrate the remarkable control over sulfur metabolism in *Lemna*, which permits both the pool sizes of sulfur amino acid intermediates and the synthesis of organic sulfur compounds to remain essentially unchanged when the plant is presented with a 3000-fold variation in the environmental concentration of sulfate. This suggests that the concentration of inorganic sulfate is probably not involved directly in regulation of methionine biosynthesis.

(3) Experiments are now under way to determine in *Lemna* whether the *de novo* synthesis of methionine from inorganic sulfate is subject to feedback control by methionine. *Lemna* was grown in the presence of either sulfate-^{35}S as a sole source of sulfur, or in the presence of sulfate-^{35}S plus exogenous methionine. If methionine exerts feedback control, then exogenous methionine should be used in preference to its *de novo* synthesis from sulfate-^{35}S, and the presence of exogenous methionine should therefore inhibit the incorporation of sulfate-^{35}S into methionine and its products. The analyses required to determine whether or not feedback control by methionine operates have not been completed. However, a somewhat unexpected finding has already been clearly demonstrated by the data on hand. In a *Lemna* plant, growing "normally" on inorganic sulfate as a sole source of sulfur, the pool of soluble methionine represents only approximately 1% of the total cellular methionine, with the balance present in protein. In plants growing at almost the same rate in the presence of added methionine, the pool of soluble methionine is increased over 50-fold, and is now a significant fraction of the total cellular methionine. This finding shows that there is no intrinsic barrier to increasing significantly the methionine content of this plant, and is encouraging in our long-

term goal of improving the nutritional quality of plants by increasing their methionine content.

Future work will be continued along the following lines:

(a) The extent of feedback control by methionine will be quantitated, and the site(s) of this control localized by determining the initial fluxes of isotope from inorganic sulfate-^{35}S into the intermediates of methionine biosynthesis. These isotope studies will be correlated with measurements of the levels of enzymes and substrates of the two pathways.

(b) Most of our studies to date on the relative importance of the two pathways and their control have been with intact plants. Already these *in vivo* studies have raised a number of interesting questions which should be persued at the molecular level. For example, the very efficient coupling between the synthesis of soluble cysteine and cystathionine that was indicated by *in vivo* studies suggests that the respective enzymes (O-acetylserine sulfhydrylase and cystathionine γ-synthase) that synthesize these intermediates are intimately associated. The molecular basis of this possible association will be examined. Our present *in vivo* studies on the regulation of the pathways are expected to pinpoint these enzymes whose catalytic and regulatory properties are deserving of intensive study.

Publication:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00981-12-LNB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Analysis of the Macromolecular Structure of Nerve Membrane During Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Tasaki	Chief	LNB NIMH
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	T. Warashina	Visiting Fellow	LNB NIMH
	R. Sandlin	Electronic Technician	LNB NIMH

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Woods Hole, Massachusetts

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	4.0	2.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The macromolecular structure of the nerve membrane involved in the process of excitation has been studied using both crab nerves and squid giant axons. In a project designed to gather information on which functional groups were involved in excitation, protein modifying reagents were applied to the exterior of crab nerves and to the interior of squid giant axons. It was found that modification of either histidine or sulfhydryl groups, located inside the axon, produced conduction block, but that modification of either of these groups or of amino groups on the external surface of the axon, had no effect on excitability. These observations led to the speculation that histidine and sulfhydryl groups, located intracellularly, must be involved in excitation, but that amino groups are not. In another project, proteins from squid giant axons were labeled covalently with a variety of radioactive compounds. By collecting the perfusate from internally perfused squid giant axons, labeling these proteins with ^{125}I-Bolton-Hunter reagent, and analyzing them using polyacrylamide gel electrophoresis, it was found that a 56,000 dalton protein was released when excitability was lost due to perfusion with a chaotropic salt. When either ^{125}I-Bolton-Hunter reagent or

^{3}H-DNFB were introduced into a squid axon, the labeling efficiency of these compounds was increased by electrically stimulating the axon. When a squid giant axon was incubated in a solution containing AT^{32}P(γ), three major protein bands were found to be phosphorylated (molecular weights: 400,000, 200,000 and 12,000). The phosphorylation of two of these proteins was sensitive to electrical stimulation.

Chemical modification of nerve membrane. In the past year we studied the macromolecular structure of the nerve membrane involved in the process of excitation, mainly by applying biochemical methods to excitable nerve fibers. Protein modifying reagents and proteolytic enzymes were applied to both crab nerves (available in Bethesda as well as in Woods Hole) and squid giant axons (available only in Woods Hole). The experimental results obtained by using these methods during the past year have given us information on which functional groups are important in nerve excitation.

A large number of protein modifying reagents (available commercially) were applied either extracellularly to crab nerves or intracellularly in squid giant axons. The amplitude of the propagated action potential was monitored so that these reagents could be classified according to their ability to block nerve conduction when applied at a low concentration. Four classes of reagents were examined: reagents which modify sulfhydryl groups, imidazole groups, methionine groups and amino groups in proteins. By applying sulfhydryl modifying reagents extracellularly to crab nerve, it was found that only those reagents with appreciable lipid solubility blocked nerve conduction, thus indicating that there exists a sulfhydryl group on the axoplasmic side of the membrane which, when modified, causes nerve conduction block. This interpretation was substantiated by observations made with sulfhydryl reagents to which the nerve membrane is impermeable. These reagents were found to block nerve conduction very effectively when introduced intracellularly into squid axons; however, when applied extracellularly they did not have much effect. The imidazole modifying reagents revealed the presence of histidine groups, located on the axoplasmic side of the membrane, which when modified, blocked nerve conduction. A methionine group was found to be accessible to cyanogen bromide testing and cleavage of the peptide bonds next to this group resulted in nerve conduction block. Finally, no nerve conduction block could be found which could be attributed to the specific modification of amino groups. This study has thus given us a picture of some of the chemical groups which are important in nerve conduction. This information is important in developing a site-specific probe which could be used in further analyzing the proteins involved in nerve conduction.

Although the electrophysiological effects of applying proteolytic enzymes to the inside of squid giant axons have been studied for years, practically none of these previous studies have taken into account the effect of the salts in the solution in which the enzymes were dissolved. We have observed that the time necessary for nerve conduction block due to intracellular application of these enzymes is dependent on the salt solution

in which it is dissolved: when dissolved in a potassium fluoride containing solution, the time necessary to block conduction was generally longer than when the enzyme was dissolved in a solution containing a more chaotropic salt such as potassium aspartate. Furthermore, the effects of these enzymes could be reversed by changing the salt solution used to perfuse the interior of the axon from a potassium aspartate containing solution to a potassium fluoride containing solution. This observation suggests that the initial nerve conduction block was in fact due to the chaotropic nature of the salt solution used to dissolve the enzyme, and that the enzymatic action hastened this process by removing some of the protein covering the sites sensitive to the chaotropic salt.

Isolation of protein from nerve membrane. In an effort at understanding the role of axonal proteins in maintaining the excitability of squid giant axons, we have developed and used, in the past year, several methods which have provided information on axonal proteins in these axons. These methods all depend on enhancing the sensitivity of conventional biochemical techniques sufficiently to be able to detect the very small amount of protein in a single squid giant axon. This was accomplished by radioactively labelling, either in situ or in vitro, the axonal proteins using a variety of commercially available reagents. In a separate series of experiments with a similar objective, the squid axons were perfused with the proteolytic enzyme pronase or with chaotropic salts, and subsequently the inner surface of the membrane was examined by scanning electron microscopy. These experiments were performed in collaboration with Dr. Metuzals.

The perfusate from squid giant axons was collected and the proteins in the perfusate were covalently labeled with ^{125}I-Bolton Hunter reagent (commercially available at a very high specific activity). The proteins were then analyzed by polyacrylamide gel electrophoresis. The propagated action potentials of the axons were monitored so that a correlation could be made between the loss of excitability and the proteins released into the perfusate. We were able to confirm, using this technique, previous work done in this laboratory in which axonal protein was labeled in situ with ^{3}H-N-ethyl-maleimide. It was shown that the strength of the chaotropic salt used in the perfusion solution determined both the amount of protein released into the perfusate and the length of time that the axon was able to survive perfusion with that particular salt solution. Furthermore, it was found that protein of about 56,000 daltons was released when axonal excitability was lost due to intracellular perfusion of a potassium chloride solution.

Two radioactive reagents, ^{125}I-Bolton-Hunter reagent and ^{3}H-dinitrofluorobenzene were used to label squid giant axons in situ by introducing these reagents into an axon. It was found that electrical stimulation of the axon during the radioactive labeling increased the labeling efficiency of the reagents, resulting in a higher specific activity of axoplasmic proteins in stimulated compared to non-stimulated axons. This increase in labeling efficiency was attributed to the increased exposure of protein sites during excitability. Furthermore, when extruded axoplasm was incubated

for 20 minutes in the presence of low concentrations of calcium, the profile of the axoplasmic proteins on polyacrylamide gel electrophoresis changed: more low molecular weight and fewer high molecular weight proteins were found. In the absence of calcium, the profile remained stable. This observation strengthened the suggestion of Gilbert et al. (Nature, 256: 586, 1975) that axoplasm contains a calcium activated proteolytic enzyme.

When a squid giant axon was incubated in artificial sea water containing $AT^{32}P(\gamma)$, three major protein bands were found to be phosphorylated: a large band at 400,000 daltons, a smaller band at 200,000 daltons and a band with a variable size at 12,000 daltons. When the axon was electrically stimulated, more of the 12,000 dalton protein and less of the 200,000 dalton protein was found to be phosphorylated compared to unstimulated axons. When cyclic AMP was introduced into an axon which was incubated in a solution of $AT^{32}P$, the phosphorylation of the 200,000 dalton protein was found to be diminished. External application of cyclic AMP had little effect on the process of phosphorylation by this method. These observations suggest that two axoplasmic proteins undergo changes during electrical stimulation. These phosphorylation studies are being persued in the hope of elucidating the relation between nerve excitation and protein phosphorylation.

Scanning electron microscope pictures of the inner surface of squid giant axons revealed an intricate fibrous structure visible only after the axon had been perfused briefly with a pronase solution. In axons treated internally with pronase until nerve conduction was suspended, a large portion of the axolemma was found to be free of the adhering protein layer. These observations strongly suggested that the layer of fibrous proteins adhering to the inner surface of the axolemma is essential for the maintenance of excitability.

Publications:

Inoue, I., Pant, H.C., Tasaki, I. and Gainer, H.: Release of proteins from the inner surface of squid axon membrane labeled with tritiated N-ethylmaleimide. J. Gen. Physiol. 68: 385-395, 1976.

Tasaki, I.: Chronaxie and transitional decrement: A review of Dr. Davis' contributions to physiology of nerve fibers. In Hirsh, S.K., Eldredge, D.H. Hirsh, I.J. and Silverman, S.R. (Eds.): Hearing and Davis: Essays Honoring Hallowell Davis. St. Louis, Washington Univ. Press, 1976, pp. 1-10.

Tasaki, I.: Physico-chemical properties of the nerve membrane. In Selegny, E. (Ed.): Charged Gels and Membranes, II. Dordrecht-Holland, D. Reidel, 1976, pp. 213-238.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00982-10 LNB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Sensory-motor Integration in the Primate Visual System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.H. Wurtz	Physiologist	LNB NIMH
	B.J. Richmond	Staff Associate	LNB NIMH
	S.J. Judge	Visiting Fellow	LNB NIMH
	J.E. Albano	Guest Worker	LNB NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For over a century scientists interested in the nervous system have postulated various mechanisms the brain uses to deal with the visual stimulation resulting from eye movements: "corollary discharge" (e.g., Helmholtz), proprioception (e.g., Sherrington) and complex visual stimulation (e.g., Gibson). We have found that the brain deals with one aspect of this problem, the elimination of visual stimulation generated by its own rapid eye movements using not one but several mechanisms. In the primary visual cortex, which we think is closely related to visual perception, we found that cells behave in ways we would expect if these cells underlie perception during eye movements. The primary mechanism here appears to be visual interaction. In the other branch of the visual system, we found that activity from oculomotor areas of the brain reduce the sensitivity of the superior colliculus cells to visual stimulation -- a potent corollary discharge. But rather than a perceptual function (which is usually postulated) we suggest that this corollary discharge eliminates spurious self-generated visual signals from reaching the oculomotor system and initiating a subsequent eye movement.

We went on to test the interaction of stationary and moving stimuli on these cells studied not during eye movements but while the monkey was fixating. The results were comparable to the results found during eye movements: the response of the cell to a moving stimulus was eliminated or attenuated by stationary stimuli falling on the receptive field before and after a rapid stimulus movement. The "masking" effect was therefore not dependent on the occurrence of eye movements but just on the interaction of the sequential visual stimuli. The classification of cells so affected and the time course of the interaction between stimuli were also investigated. Thus we think we have found an answer at both the behavioral and physiological level to the question of why we do not see during eye movements.

The visual pathway from the eye to the brain in both man and monkey has a second branch in addition to the one discussed above. This pathway extends directly from the eye to the superior colliculus lying on the roof of the midbrain. Here Robinson and Wurtz (as summarized in previous annual reports) found that cells were actively suppressed during eye movements and that this suppression eliminated the response of cells to visual stimuli present during eye movements. This suppression did not result from any visual masking as is the case in the visual cortex. The question we (B.J. Richmond and R.H. Wurtz) answered this year was whether the suppression of activity is due to the sensory information flowing into the brain during an eye movement from the eye muscles (proprioception), or whether the suppression is due to a message from within the nervous system which is a corollary of the impulses sent to the muscles causing the eyes to move.

Our plan in these experiments was to remove the proprioceptive input by paralyzing the eyes. If the suppression resulted from proprioception, the suppression should disappear during paralysis since such proprioception would be eliminated. We first isolated a cell which showed a suppression of discharge rate when the monkey made a saccadic eye movement. We then paralyzed the eyes by using 1% xylocaine as a local anaesthesic (a retro-bulbar block); complete paralysis of the eyes was achieved (less than 3 minutes of arc residual movement) lasting 15-20 minutes. The occurrence of attempted eye movements was indicated by the discharge of cells recorded from an electrode previously implanted in the motor nucleus connected to the eye muscles (medial rectus division of the oculomotor nucleus). The discharge of 17 cells which showed the suppression of background activity with normal eye movements continued to show the suppression during the complete paralysis. For several cells, the suppression was observed before, during, and after the period of paralysis. Since the suppression is present when no eye movement occurs, no signal related to the eye movement (including proprioception) can be causing the suppression: instead the suppression must have its origin in that part of the nervous system related to the intention to move the eyes. This is the first time such a corollary discharge has been shown to modify so powerfully the visual processing by cells in the visual system of mammals. This potent "corollary discharge" is thus used to eliminate the signal of image movement on the retina secondary to eye movements from the response of some cells in the superior colliculus. We think this corollary discharge has nothing to do with visual perception but rather eliminates spurious signals to the oculomotor system about movement generated by the animal's own eye movements.

Publications:

Künzle, H., Akert, K. and Wurtz, R.H.: Projection of area 8 (frontal eye field) to superior colliculus in the monkey. An autoradiographic study. Brain Res. 117: 487-492, 1976.

Mohler, C.W. and Cechner, R. Saccadic suppression in the monkey. Vision Res. 15: 1157-1160, 1975.

Mohler, C.W. and Wurtz, R.H.: Role of striate cortex and superior colliculus in visual guidance of saccadic eye movements in monkeys. J. Neurophysiol 40: 74-94, 1977.

Wurtz, R.H., Mohler, C.W. and Robinson, D.L.: Visual motor integration in monkey superior colliculus. In Creutzfeldt, O. (Ed.): Afferent and Intrinsic Organization of Laminated Structures in the Brain. Berlin Heidelberg Springer-Verlag, 1976, pp. 551-555.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01031-09 LNC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Conversion of Phenylalanine to Tyrosine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Sheldon Milstien	Chemist	LNC NIMH
	John Donlon	Visiting Fellow	LNC NIMH
	Norman Chang	Senior Staff Fellow	LNC NIMH
	George Summer	Guest Worker	LNC NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.3	4.3	1.0

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new variant form of phenylketonuria (PKU) was found. This disease is due to a deficiency of the pterin cofactor. An in vivo assay for phenylalalanine hydroxylase activity performed on the patient with this form of PKU showed that he has only about 5% of the normal activity.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this research project is the detailed description of the complex enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components of the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase, and tetrahydrobiopterin.

A related objective is to use this basic knowledge of the phenylalanine hydroxylase system to increase our understanding of the disease, phenylketonuria, (PKU), as well as its variants. Specifically, the goal is to devise methods of detection and therapy for these diseases, as well as to understand their pathogenesis.

In the reports from the last two years, results of our biochemical description of the newly discovered variant of PKU, hyperphenylalaninemia (HPA) due to dihydropteridine reductase deficiency were summarized. In our original report of this disease, we proposed that the neurological deterioration that characterizes this disease was due to blocks in the biosynthesis of the neurotransmitters, dopamine, norepinephrine and serotonin. We also recommended that administration to these patients of the two precursor substances whose synthesis was probably blocked, DOPA and 5-hydroxytryptophan, would be a rational therapy for the disease. This therapy has now been tried on about 6 patients. The results indicate that when initiated early enough, the therapy is effective. Significantly, without therapy the disease appears to be lethal. To date, all of the treated patients are alive.

During the last year, we have characterized a second new disease, hyperphenylalaninemia due to a deficiency of biopterin. Biochemical analysis on a biopsy liver sample showed that the hydroxylase and dihydropteridine reductase are present in normal or high-normal amounts, whereas the level of pterin cofactor is only 5 to 10% of normal. The affected child, who is now 6 years old, is mentally retarded and shows poor motor development.

Since our earlier work showed that tetrahydrobiopterin is an essential component of tyrosine and tryptophan hydroxylases, we believe that the deterioration in this patient is due to a deficiency in the neurotransmitters, dopamine, norepinephrine and serotonin and have proposed that the same therapy, i.e., administration of DOPA and 5-hydroxytryptophan, should be effective in this disease.

Just as with hyperphenylalaninemia due to reductase deficiency, a method of early diagnosis is essential for the early treatment of this variant form of PKU. To this end, we have set up during the last year a microbiological assay for biopterin and related compounds that is based on the requirement of the organism, Crithidia fasiculata, for an unconjugate

pterin for maximum growth. With this assay, we have shown that the amount of biopterin excreted in this patient's urine is far below normal. These results indicate that a determination of urinary biopterin levels should be able to provide a means for the early diagnosis of this new disease.

We have previously described an in vivo assay for phenylalanine hydroxylase that had been validated in rats. The method is based on the liberation of tritiated water from ring-tritiated phenylalanine during its conversion to tyrosine. The amount of tritiated water released into body fluids (such as blood or urine) in a given period of time is a quantitative measure of the rate of phenylalanine hydroxylation.

We have tested this phenylalanine hydroxylase assay in humans for the first time. A small amount of tritiated phenylalanine was administered to the patient with hyperphenylalaninemia due to biopterin deficiency. Radioactivity measurements on blood samples confirmed the finding that he has a block in phenylalanine metabolism and showed that his rate of conversion of phenylalanine to tyrosine is only about 5% of normal. This defect is in accord with what we expected from our earlier finding that the biopterin content in this patient's liver is only 5 to 10% of normal.

We expect to try this tritium-release assay on additional patients in the future.

Publications:

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Kaufman, S.: The phenylalanine hydroxylating system in phenylketonuria and its variants. Biochem. Med. 15: 42-54, 1976.

Abita, J.-P., Milstien, S., Chang, N. and Kaufman, S.: In vitro activation of rat liver phenylalanine hydroxylase by phosphorylation. J. Biol. Chem. 251: 5310-5314, 1976.

Milstien, S., Abita, J.-P., Chang, N. and Kaufman, S.: Hepatic phenylalanine 4-monooxygenase is a phosphoprotein. Proc. Natl. Acad. Sci. USA 73: 1591-1593, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01032-09 LNC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Emanuel Diliberto	Sr. Staff Fellow	LNC NIMH
	Robert Pollock	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this work is the detailed description of the hydroxylation reactions that are involved in the biosynthesis of the neurotransmitters, dopamine and norepinephrine. Recently, our emphasis has been on dopamine β-hydroxylase and two enzymes involved in the synthesis of tetrahydrobiopterin, dihydropteridine reductase and dihydrofolate reductase. Dihydrofolate reductase activity has been found in brain and shown to reduce dihydrobiopterin. The ability of dihydropteridine reductase to function in folate metabolism has also been demonstrated. With regard to dopamine-β-hydroxylase, the emphasis has been to study the mechanism for catechol oxidation by this enzyme.

PHS-6040
(Rev. 10-76)

Project Description

The conversion of tyrosine to norepinephrine requires several enzymes and cofactors including tyrosine hydroxylase, dopamine- β-hydroxylase, dihydropteridine reductase and tetrahydrobiopterin. Dihydrofolate reductase can also be considered a member of this list, since it is necessary for biosynthesis of tetrahydrobiopterin.

The last step in the synthesis of tetrahydrobiopterin is the reduction of 7,8-dihydrobiopterin, catalyzed by dihydrofolate reductase. Other laboratories have presented evidence that brain can synthesize its own tetrahydrobiopterin but it had been previously reported that brain lacks dihydrofolate reductase. Work in this laboratory has now demonstrated the presence of this enzyme activity in brain and has shown that 7,8-dihydrobiopterin can be reduced to tetrahydrobiopterin by brain extracts. The ability of brain to synthesize this cofactor and, thus, not be dependent on peripheral sources is important to catecholamine synthesis in brain since the tetrahydrobiopterin concentration is thought to limit the activity of tyrosine hydroxylase, the enzyme catalyzing the rate limiting step in catecholamine biosynthesis.

Dihydropteridine reductase functions in brain to regenerate tetrahydrobiopterin from the quinonoid-dihydrobiopterin, product of tyrosine and tryptophan hydroxylation. Although dihydropteridine reductase is widespread in mammalian tissues, other possible roles for it have been found. This laboratory has investigated the possible function of this enzyme in folate metabolism since it is known that tetrahydrofolate is readily oxidized non-enzymically to quinonoid-dihydrofolate, a substrate for this enzyme. We have found that dihydropteridine reductase is necessary for full activity of the tetrahydrofolate-dependent serine to glycine conversion in brain homogenates. This evidence that dihydropteridine reductase functions in folate metabolism helps to explain the abnormally low folate content we have observed in an atypical phenylketonuric patient that lacks dihydropteridine reductase.

Another major focus of research during the last year has been a detailed study of the mechanism of catechol oxidation by dopamine- β-hydroxylase. Dopamine- β-hydroxylase, a mixed-fuction oxidase, catalyzes the ascorbate-dependent oxidation of dopamine to norepinephrine; this is the final step in the biosynthesis of the neurotransmitter, norepinephrine. Ascorbate functions in this system by reducing enzyme-bound Cu^{2+} to Cu^{1+}; this reduced form of the enzyme then reacts with the substrate and molecular oxygen to form the hydroxylated product and to regenerate the oxidized form of the enzyme. In the absence of β-hydroxylating substrate, after a stoichiometric oxidation of ascorbate in the formation of reduced enzyme, no further enzyme-dependent oxidation of ascorbate occurs. An earlier observation, that provided a clue to this mechanism of β-hydroxylation, was that, in the absence of substrate, the enzyme catalyzed the oxidation of catechol. It was shown by sucrose gradient ultracentrifugation, heat

denaturation and antibody precipitation that the same protein catalyzed both the substrate β-hydroxylation and the "substrate-less" or "catechol oxidase" reactions. The essential difference between the ability of dopamine-β-hydroxylase to catalyze the oxidation of catechol and not ascorbate probably corresponds to the stability of the reduced form of the enzyme produced by catechol and ascorbate, respectively. To test the possibility that catechol might irreversibly transform the enzyme into a more general oxidase, dopamine-β-hydroxylase was pretreated with catechol. After removal of excess catechol by gel filtration, the enzyme was tested for its ability to catalyze the oxidation of ascorbate; no enzyme-dependent oxidation of ascorbate was found. Earlier observations lead to the proposal of several models to account for catechol oxidation by this enzyme. The product of enzymic catechol oxidation, o-benzoquinone, was thought to be involved in the reoxidation of the reduced form of the enzyme. Since catalytic reoxidation of the reduced enzyme by o-benzoquinone could not be demonstrated, this model for catechol oxidation by dopamine-β-hydroxylase did not appear to be plausible. Superoxide anion, which is generated during the spontaneous (and possibly the enzymic oxidation) of catechol, was shown to reduce dopamine-β-hydroxylase, making it unlikely to be involved in the reoxidation of the reduced form of the enzyme. More recent experiments suggest that catechol may be involved in a single electron transfer to dopamine-β-hydroxylase in the formation of a partially reduced enzyme. This form of the enzyme may interact with molecular oxygen to undergo spontaneous reoxidation. More recent experiments suggest that the reason that the hydroxylase has "catechol oxidase" activity and not "ascorbate oxidase" activity is that the reduced form of the enzyme produced by catechol and ascorbate are not identical. Our evidence suggests that, in contrast to ascorbate, catechol reduces the enzyme to a form that is auto-oxidizable in the presence of molecular oxygen.

Publications:

Hoeldtke, R. and Kaufman S.: Bovine adrenal tyrosine hydroxylase. Purification and properties. J. Biol. Chem. 252: 3160-3169, 1977.

Katz, I., Lloyd, T. and Kaufman, S.: Studies on phenylalanine and tyrosine hydroxylation by rat brain tyrosine hydroxylase. Biochim. Biophys. Acta 445: 567-578, 1976.

Kopin, I.J., Kaufman, S., Viveros, H., Jacobowitz, D., Lake, C.R., Ziegler, M.G., Lovenberg, W. and Goodwin, F.K.: Dopamine-β-hydroxylase. Basic and clinical studies. Annals of Internal Medicine 85: 211-223, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01034-09 LNC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Biochemical Basis of Skeletal Muscle Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Philip Fleckman	Medical Officer (Res.)	LNC NIMH
	Herman Vandenburgh	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. Present topics of investigation include: 1) The relationship between skeletal muscle and connective tissue in hypertrophy; 2) the relationship between compensatory skeletal muscle hypertrophy and endurance training; 3) localization of phosphoinositide changes in hypertrophy using radioautography; and 4) the relationship between stretch-induced hypertrophy and muscle protein synthesis and degradation.

PHS-6040
(Rev. 10-76)

Project Description:

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. The animal model used in this project is the hypertrophy of the soleus and plantaris muscles which occurs when the synergistic gastrocnemius muscle is incapacitated by tenotomy of the achilles tendon. The contralateral sham-operated leg serves as control.

Previous work in this laboratory has demonstrated that compensatory skeletal muscle hypertrophy is accompanied by connective tissue cell proliferation and that the enhanced RNA synthesis associated with hypertrophy is localized to these proliferating non-myofiber elements. This proliferation is not essential for hypertrophy since DNA synthesis can be almost completely inhibited without significantly affecting muscle hypertrophy. Also, collagen content is the same in hypertrophied and control muscle. These findings suggest that increased connective tissue metabolism is not essential for the initiation of muscle hypertrophy. Specific myofibrillar protein changes in hypertrophying muscles in which DNA synthesis is inhibited have been examined and do not differ from normal hypertrophied muscle. In using these procedures, a new and more specific criterion for skeletal muscle hypertrophy, i.e., increase in myofibrllar protein, is evolving.

Endurance-trained muscles have metabolic alterations which differ from those seen in hypertrophied muscles. For example, enzymes involved in the activation, transport, and oxidation of fatty acids, enzymes of the citrate cycle, and enzymes of the respiratory chain have been shown to be markedly increased in activity in muscles of endurance-trained animals. This results in an increased capacity to oxidize fatty acids and pyruvate and a decrease in anaerobic glycolysis. To investigate whether these two different types of metabolic adaptations of muscle are mutually exclusive, animals have been endurance trained and then tenotomized. Under the conditions studied, endurance training has no apparent affect on subsequent muscle hypertrophy. These results help in focusing our hypotheses on the mechanism of skeletal muscle hypertrophy, e.g., one hypothesis is that hypertrophy results from the accumulation of a metabolite (secondary to increased work) which stimulates muscle growth. If such an accumulation occurs, it is not prevented by endurance training.

Work in this laboratory has shown increased incorporation of myoinositol into inositol phospholipid early in muscle hypertrophy. A radioautographic technique has been developed to localize this inositol effect. Preliminary data indicate that, unlike the increase in RNA synthesis, the increase in inositol incorporation takes place in the muscle cells and may therefore mark an early event in the biochemical changes of the hypertrophying muscle.

Other investigators have hypothesized that compensatory skeletal muscle hypertrophy is due to stretching of the hypertrophying muscle, which leads to increased tension and increased work. Investigation of the relationship between stretch and myofibrillar protein synthesis and degradation are in progress using a technique developed in this laboratory of stretching monolayers of chick embryonic skeletal myotubes in vitro.

Publications:

Jablecki, C., Dienstag, J. and Kaufman, S.: ^{3}H-inositol incorporation into phosphatidyl-inositol in work-induced growth of rat muscle. Am. J. Physiol. 232: E324-329, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01035-09 LNC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Process of Lysogeny

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Nash	Medical Officer (Research)	LNC NIMH
OTHER:	Yoshiko Kikuchi	Visiting Fellow	LNC NIMH
	Carol Robertson	Biologist	LNC NIMH

COOPERATING UNITS (if any)
LMG, NICHD; LMB, NCI; LMB, NIAMDD: Dept. of Molecular Biology & Biochemistry, Univ. of California, Irvine, Ca. 92717; Dept. of Microbiology, Univ. of Michigan Medical School, Ann Arbor, Mi. 48109.

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biochemistry of the site-specific recombination that integrates the genome of bacteriophage λ into that of its E. coli host has been studied. Supertwisted DNA has been discovered to be the obligate substrate for this reaction in cell free extracts. The supertwists are introduced into circular DNA by the action of a newly discovered enzyme, DNA gyrase. This enzyme has been purified and characterized; its role in recombination appears to be the same in whole cells as in the cell free system. An additional recombination enzyme, the product of the viral int gene has been purified and characterized. It demonstrates specific binding to substrate DNA and, together with several bacterial proteins, carries out the breakage and reunion of DNA.

Project Description:

Lysogeny defines that portion of the life cycle of a bacterial virus that results in the stable survival of both viral and bacterial genomes. The altered bacterium, called a lysogen, retains the viral chromosome in novel union with the bacterial chromosome. The association of the viral and bacterial genomes is achieved through a genetic recombination that integrates the viral DNA into the linear continuity of the bacterial DNA. Integration of DNA is a process found in nature in organisms as diverse as bacteria and man. It appears to be a frequently used mechanism both for the addition of new genetic information to an organism and for the rearrangement of existing information. Such additions and rearrangements are thought to be essential to processes like the creation of genetic variability between individuals, the formation of tumors, and the biogenesis of antibodies.

For several years this laboratory has been investigating the biochemistry of one such integrative recombination - the insertion of the DNA of bacteriophage lambda into the chromosome of E. coli. These studies have provided the first detailed insight into the mechanism of a naturally occurring recombination. Two aspects of this problem have been recently studied in detail - the formation of supertwisted DNA and its use in integrative recombination and the characterization of the enzymes which carry out the actual recombination event.

Supertwisted DNA substrate is required for integrative recombination in cell free extracts. Linear DNA or circular DNA with interruptions can only serve as substrate if they are first converted to covalently closed circles. Covalently closed circles that have no supertwists serve as efficient substrates only if the cofactor ATP is added to in vitro reactions; supertwisted DNA substrates are effective in the presence or absence of ATP. These observations indicated that cell free extracts prepared for integrative recombination contain an ATP-dependent activity that converts relaxed closed circles into supertwisted circles. We have succeeded in isolating an enzyme with such activity. The enzyme called DNA gyrase, is present in uninfected E. coli and has been partially purified and characterized as a protein of approximate molecular weight of 140,000 daltons. In the presence of magnesium and ATP, the enzyme catalyzes the introduction of negative superhelical turns into closed circular DNA; the final product strongly resembles naturally occurring circular DNA. This enzymatic reaction is a novel one; it involves the breakage, unwinding, and resealing of the DNA strands in a concerted reaction. The enzyme therefore serves as a chemo-mechanical transducer, presumably converting the chemical energy of ATP hydrolysis into mechanical work of unwinding a DNA helix.

In living bacteria DNA gyrase is an important enzyme. Recent work from the laboratories of M. Gellert and J. Tomizawa have shown that several antibiotics kill bacteria as a result of specific inhibition of DNA gyrase. Using such antibiotics we have shown that DNA gyrase activity is required

for integrative recombination in whole cells as it is in cell free extracts Moreover, other workers have shown that the DNA gyrase catalyzed production of supertwisted DNA is probably essential to the processes of replication and transcription of DNA in bacteria.

What happens to supertwisted substrate DNA during a recombination reaction? Even if DNA gyrase activity is blocked, we find that the recombinant products have retained virtually all of the supertwists present in the substrate. This means that during the process of breakage and realignment of the substrate DNA, broken DNA intermediates are held fixed prior to resealing. In order to expidite the analysis of the enzymes involved in this conversion of supertwisted substrate DNA to a recombinant product, we have developed a rapid, quantitative assay for integrative recombination in vitro. This method assays the recombination dependent rearrangement of substrate DNA to a form which is uniquely trapped on nitrocellulose filters. Using this assay we have achieved an extensive purification of Int - the virus coded product required for integrative recombination. Our best preparations are more than 50% pure and contain approximately 2% of the initial Int activity found in cell free extracts. We calculate that Int makes up only 0.01% of the soluble protein of a cell, accounting for about 100 molecules per cell. Recent studies by this and other laboratories have revealed an unsuspected control system for the expression of the int gene in whole cells. Using this information we are devising methods to increase the concentration of Int in whole cells in order to improve the amount recovered by our purification scheme.

Purified Int has been shown to be free of contaminating nucleases, polymerases, and ligases; in fact no enzymatic activity is observed with substrate DNA unless appropriate host recombination components (see below) are added. Purified Int does bind tightly to DNA and exhibits a marked preference for DNA containing the sites at which recombination occurs. This specific interaction between a recombination enzyme and its substrate will be the subject of a detailed future study.

The final component of the integrative recombination reaction to be studied are bacterial proteins that are used together with Int to recombine supertwisted substrate DNA. Like DNA gyrase, these components may play several roles in the physiology of the cell and be exploited by the viral integration system as needed. In collaboration with the laboratories of Drs. D. Friedman, R. Weisberg, and D. Wulff we have identified genes for two of these components. Two classes of mutants of E. coli that are defective in integrative recombination in vivo were examined as the source of extracts for recombination in vitro. Both classes proved to be normal in production of Int but defective in the overall reaction. Since the inactive extracts from each of the two classes can be combined to provide an active mixture, each mutant class must define a gene that encodes a product playing a direct role in integrative recombination. The isolation, purification, and characterization of these products is ongoing.

Publications:

Mizuuchi, K. and Nash, H.A.: Restriction assay for integrative recombination of bacteriophage λ DNA in vitro: Requirement for closed circular DNA substrate. Proc. Natl. Acad. Sci. USA 73: 3524-3528, 1976.

Gellert, M., Mizuuchi, K., O'Dea, M.H. and Nash, H.A.: DNA gyrase: An enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA 73: 3872-3876, 1976.

Nash. H.A., Mizuuchi, K., Weisberg, R.A., Kikuchi, Y. and Gellert, M.: Integrative recombination of bacteriophage λ - The biochemical approach to DNA insertions. In Shapiro, J., Bukhari, A. and Adhya, S. (Eds.): Plasmids, DNA Insertion Elements, and Episomes. New York, Cold Spring Harbor Laboratory, 1977, in press.

Williams, J.G.K., Wulff, D.L. and Nash, H.A.: A mutant of Escherichia coli deficient in a host function required for phage lambda integration and excision. In Shapiro, J., Bukhari, A. and Adhya, S. (Eds.): Plasmids, DNA Insertion Elements, and Episomes. New York, Cold Spring Harbor Laboratory, 1977, in press.

Court, D., Adhya, S., Nash, H. and Enquist, L.: The phage integration protein (Int) is subject to control by the cII and cIII gene products. In Shapiro, J., Bukhari, A. and Adhya, S. (Eds.): Plasmids, DNA Insertion Elements and Episomes. New York, Cold Spring Harbor Laboratory, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01036-05 LNC

PERIOD COVERED
July 1, 1976 through June 30, 1977

TITLE OF PROJECT (80 characters or less)
Heparin-Induced Conformations: Model for Intracellular Polysaccharide-Protein Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Audrey L. Stone Research Chemist LNC NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Physicochemical forces which sequester cell solutes from soluble form to organized structures are investigated from the standpoint of molecular conformations of components and interacting species. Earlier results showed that ordered, negative charges (e.g., in glycosaminoglycans) have profound and specific ordering effects on amine cations. The current project studies polyanions inducing conformational constraints in otherwise non-ordered cationic polypeptides. A variety of purified polysaccharides (PS) examined for conformation-promoting effects on poly-L-lysine showed little specificity; all with sufficient anionic density promoted a high degree of α-helical conformation. Investigation of specificity dependent upon peptide structure revealed that interactions of heparin (and other polyanions) with copolymer L-lysine:L-tyrosine promote the β-protein structure, while those with copolymer L-lysine:L-phenylalanine induce α-helical order. That the nature of conformational changes in protein models due to interaction with heparin depends upon constellations of amino acids and their proclivity for α-helix or β-structures is significant in the molecular basis of multianion action in granule formation and enzyme activation.

Project Description:

Anionic polysaccharides (glycosaminoglycans (GAG)) are intimately involved in the biological formation and function of inter- and intra-cellular matrices and some, such as heparin, are known to stimulate or inhibit certain enzyme systems. Intracellular storage regions, or discrete granules, occur in vario organs of many species, including synaptic processes of catecholaminergic neurons. Physicochemical forces which sequester cell solutes from a soluble form to a relatively insoluble, organized structure can be investigated from the standpoint of the molecular conformations of the components and of their interacting species. Investigation of the macromolecular structure of anionic polysaccharides in solution was undertaken at the onset of this project in the early 60's with the subsequent elucidation of helical conformation in heparin and other GAG in solution.

The histology, chemical composition, and explosive release of the histamine containing granule of the mast cell were well described at that time; therefore, the heparin-histamine system was used as a conceptual model for storage and release of small potent biologically active molecules (although other multi-anion/cations apply in different systems). Thus, the conformational aspects of heparin/histamine (or cationic aromatic dyes) binding were studied using optical methods of absorption and circular dichroism (CD). The results of those early studies were the first to demonstrate ultraviolet asymmetric absorption bands for heparin and other GAG. Results with the cationic amine dyes led to the first reports of helical structures for these polysaccharides in solution. Both methods showed that GAG comprise two conformational classes in solutions near pH 7. Specific conformations were elucidated for related plant polysaccharides. Direct X-ray analysis recently performed in other laboratories now confirm the various helical patterns predicted by the early reports (M-NC-21 1964-67). The results also showed that ordered negative charges have profound and specific ordering effects on amine cations.

The current project extends these basic molecular findings to the study of a third component of inter- and intra-cellular matrices, e.g., the polypeptide. As with small amines, acid polysaccharides (PS) play a role in inducing conformational constraints in cationic polypeptides. The major objective of the current project is to quantitatively determine the conformation-altering effect of various anionic PS, and to determine possible specificity of this effect on the basis of PS conformations revealed earlier in this project. These model interactions will be applied directly to studies of enzyme activation and sequestration by heparin, and related biopolymers, in simulated matrix conditions.

The methods are measurements of molecular asymmetries by extrinsic and intrinsic Cotton effects using the Cary Model 6001 circular dichroism apparatus. The metachromatic reactions developed earlier in the project (measured spectrophotometrically and by circular dichroism) provide a quantitative measure of the degree of interactions with anionic sites. Computer models for determination of the proportion of α-helical, β- or non-ordered structure in the polypeptide reveal the degree and kind of conformation induced by a given PS.

Studies of striatal tyrosine hydroxylase utilize dissection of mouse striatum and partial purification by means of a one-step separation by affinity chromatography.

Results using purified PS from plant, bacterial and mammalian sources, well-characterized as to the chemical nature of the anionic groups and the charge density, showed (Z01 MH 01036-04 LNC) that the non-ordered form of PLL was altered markedly by a strong electrostatic interaction with polysulfates, polyuronates, and mixed polyanionic groups on a homo- or complex polysaccharide backbone. Furthermore, there was little specificity in the interactions since both classes of GAG and all PS promoted a high degree of α-helical conformation in PLL. Subsequent studies revealed that mixed polypeptides show specificity of interaction with PS depending upon the nature of the non-basic amino acid residues. Heparin induces α-helix in L-lysine copolymer with L-phenylalanine (PLP) while copolymer L-lysine:L-tyrosine (PLT) forms the β-protein structure. Metachromatic measurements showed that the stoichiometry of the reaction was 1-to-1 with positive lysyl residues and negative heparin charges for the poly(L-lysine:L-tyrosine) and close to 1-to-1 with the poly(L-lysine:L-phenylalanine). The specificity of the biological functions of heparin and other GAG, therefore, may emerge to be due to the capacity to alter the conformations of specific amino acid sequences in a given protein, rather than to solely electrostatic interactions. Methods for looking at the stoichiometry and interactions of certain specialized heparan sulfates with PLL, PLT, and PLP have been developed and indicate that this glycosaminoglycan also interacts strongly with lysine containing polypeptides.

Affinity chromatographic technique for a single passage of striatal extract using heparin fixed to Sepharose 4B showed that a low degree of substitution (<1 mg/ml) was inadequate to obtain significant increases in specific activity of tyrosine hydroxylase. Methods were developed which showed that heparin fixed to the Sepharose 4B bead was available as a polyanion and could be measured by interaction with methylene blue under our conditions. Furthermore, a 3 to 4 fold increase in the amount of heparin substituted on activated Sepharose 4B was achieved by subsequent modification of the reaction conditions.

The significance of molecular conformations of organized multianions (such as the glycoaminoglycans) in brain function and mental health was not apparent to most ten years ago. However, it was clear then that the structure of inter- and intra-cellular matrices through which neurotransmitters pass, must play a role. The early results demonstrated that small cations were held in specific and highly ordered fashion by various polyanions, described a third parameter of the metachromatic reaction, and set down general techniques for measuring the above kinds of interaction. The findings with cationic polypeptides that such complexes can regulate polypeptide conformation, depending upon the nature of the amino acids neighboring cationic residues, may have direct bearing on the biological aspects of amine storage in the CNS.

Another visible role for basic studies of molecular conformations is seen, for example, in the activation of certain enzymes by heparin. Tyrosine hydroxylase, the enzyme responsible for DOPA formation, is known to occur in both soluble and insoluble forms, of which the former is markedly stimulated by

heparin. The significance of our studies on the conformational aspects of heparin-polypeptide interactions lies in the quantitative measures of the stoichiometry, which permit the proposal of a molecular model to explain, for example, the activation of tyrosine hydroxylase, and a possible mode of regulation of this enzyme, natural as well as pharmacological. Our studies define a rational approach to selecting agents which might affect this enzyme system in a predicted manner.

A more immediate significance relates to the effect of heparin on the activity of antithrombin, the biological inhibitor of the enzyme thrombin. The specificity for heparin in this anticoagulation role remains an important question. Recent models show that electrostatic interaction of heparin with lysine residues on antithrombin are involved in the ultimate conformational change of the inhibitor but do not describe the bimolecular interaction. Our studies demonstrate experimentally that specificity for the biological effect may be due to small specific amino acid sequence in the protein, rather than the current model which assumes that heparin interacts with two lysyl residues separated from one another and exposed on the inhibitor surface. The stoichiometry of the reaction of heparin with PLL and the knowledge of the repeating distances on the heparin and in a PLL α helix enables us to propose that the biological interaction involves molecular groove of the protein rather than on its surface. Thus, molecular properties of a putative heparin substitute or fraction should be considered in this regard.

The proposed course of the project is a continuation of the basic studies of glycosaminoglycan-polypeptide interactions, with the emphasis on application to the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans of the CNS. We are looking at heparin fractions and specific heparan sulfates in regard to conformational alterations in polypeptide models, and the effect of certain cationic peptides on these interactions. Partially purified striatal tyrosine hydroxylase obtained in a one-step affinity column will be used in enzyme regulation studies. The techniques have been applied to myelin basic protein in collaboration with Dr. Marian Kies, and this will be continued. Unless we would be able to obtain a spectropolarimeter in our laboratory, further experiments will be conducted in other laboratories at NIH due to 1) an arbitrary change in accessibility of the NIMH spectropolarimeter preventing proper overseeing in the care and maintenance of this instrument for serious scientific use, and 2) deterioration of the optics of this instrument.

Publications:

Stone, Audrey L.: Circular dichroism and optical rotatory dispersion in polysaccharide structural analysis: Intrinsic and extrinsic Cotton effect. In Whistler, R.L. and Bemiller, J.N. (Eds.): Methods in Carbohydrate Chemistry Vol. VII. New York, Academic Press, Inc., 1976, pp. 120-141.

Stone, Audrey L.: Optical conformation of heparin and heparin complexes with cationic dyes, amines, and protein models. Fed. Proc. 36, 101-106, 1977.

Stone, Audrey L. and Epstein, Paul: The aggregation of basic polypeptide residues bound to heparin. Biochim. Biophys. Acta 497, 298-306, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01037-09 LNC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of the Cell Membrane in Cellular Organization, A Molecular Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Neville, Jr.	Chief, Sect. on Biophys. Chem.	LNC NIMH
OTHER:	Ta-Min Chang	Senior Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

National Institute of Arthritis, Metabolism and Digestive Diseases

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Biophysical Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein hybrids containing the active fragment of diphtheria toxin and another receptor specific binding protein. Such artificial protein hybrids may have value as a new class of pharmacologic reagents.

PHS-6040
(Rev. 10-76)

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. This phenomenon has been observed with several toxic macromolecules of bacterial and seed origin which share common features. Each macromolecule consists of two polypeptide chains crosslinked by a disulfide bond. The A chain or active chain catalytically inhibits protein synthesis inside the cell. The B chain binds toxin molecules to a specific cell surface receptor. Once bound the toxin or at least the A chain, through unknown mechanisms, gains entry to the cell cytosol and protein synthesis is inhibited. A and B chains alone are non-toxic for cells; however, their separate binding and catalytic properties can be measured. At least three toxins, diphtheria, abrin and ricin fit the above generalities.

We have studied this phenomenon of receptor mediated protein entry by developing methods to construct artificial protein hybrid molecules. These hybrid molecules may be considered as structural analogues of toxin molecules in which the original binding chain has been substituted by another protein displaying different binding specificities. The questions asked in this study are whether all receptors participate in this phenomenon or whether receptor mediated entry is limited to a class of receptors displaying unique properties. Also, there is the question of whether entry is the sole functi of the B chain or whether the A chain is also involved. If entry is a unique function of the B chain, then hybrid molecules with competent B chains will direct entry of the hybrid A chain. Such hybrids would have vast biologic potential. Since varying cell types display varying surface membrane receptors, it should be possible, by choosing the right B chain to construct hybrids which are cell type specific. Such hybrids would constitute an entirely new class of pharmacologic reagents. An obvious application would be cell type specific cancer chemotherapy.

The artificial hybrid diphtheria toxin fragment A-human placental lactogen (toxin A-hPL) has been constructed and purified. This hybrid does not effect protein synthesis in mammary gland cells carrying the lactogenic receptor.

In order to study the fate of our hybrid protein after it interacts with a receptor carrying cell, we have studied the transport of toxin A-hPL from the serum to milk compartment of lactating rabbits. Two facts emerge: 1. The hybrid is cleaved to the subunit molecular weight. 2. Toxin A enzymatic activity is lost. Both of these phenomena could account for failu to achieve inhibition of protein synthesis in mammary gland cells. We are now synthesizing hybrids using: 1. a non-cleavable cross-linking bond; and 2. incorporating structures to protect the enzymatic active site.

Publications:

Chang, Ta-Min and Neville, D.M., Jr.: Artificial hybrid protein containing a toxic protein fragment and a cell membrane receptor-binding moiety in a disulfide conjugate. 1. Synthesis of diphtheria toxin fragment A-S-S-human placental lactogen with methyl 5-bromovalerimidate. J. Biol. Chem. 252: 1505-1514, 1977.

Chang, Ta-Min, Dazord, A., and Neville, D.M., Jr.: Artificial hybrid protein containing a toxic protein fragment and a cell membrane receptor-binding moiety in a disulfide conjugate. 2. Biochemical and biological properties of diphtheria toxin fragment A-S-S-human placental lactogen. J. Biol. Chem. 252: 1515-1522, 1977.

Roth, J., Neville, D.M., Jr., Kahn, R.C., and Gorden, P.: Hormone resistance and hormone sensitivity (editorial). New Eng. J. Med. 296: 277-278, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01038-06 LNC

PERIOD COVERED
July 1, 1976 through June 30, 1977

TITLE OF PROJECT (80 characters or less)
Development of Mutant Mice for Phenylketonuria and Dihydropteridine Reductase Insufficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Audrey L. Stone Research Chemist LNC NIMH

COOPERATING UNITS (if any)
Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

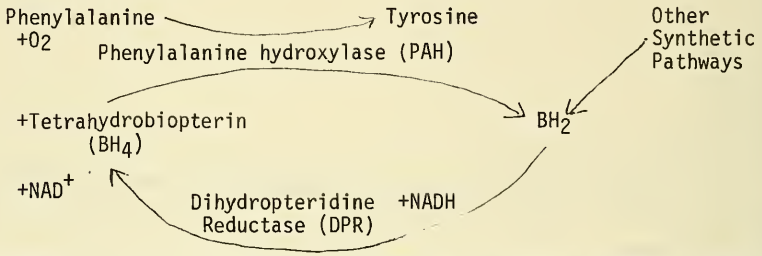
SUMMARY OF WORK (200 words or less - underline keywords)

The project is a small program to develop a murine model for PKU and/or related diseases. Another objective is the elucidation of regulatory mechanisms in the expression of phenylalanine hydroxylation (PH). Criteria for a putative deficiency in PH were established: plasma phenylalanine levels, plasma phenylalanine-to-tyrosine (P/T) ratio, and hepatic levels of dihydropteridine reductase (DPR). Early results indicating that hepatic DPR in mice was not in great excess were contrary to generally accepted hypotheses. DPR insufficiency was expressed by mild symptoms of deficiency in PH. These findings pointed out inadequacies of standard programs for detecting heterozygote mouse PKU mutants. Murine DPR insufficiency was studied using mildly elevated P/T as a criterion. Putative mutant families were developed, but infertility and/or selective survival led to a loss of viable abnormal offspring at F4 or higher. Hepatic enzyme and cofactor patterns differed among the families, a reflection of the complex nature of PH. Current work elucidates the PH system in mice, shows the relationship of in vivo elevated P/T to in vitro measurable hepatic phenylalanine hydroxylase (PAH) using parachlorophenylalanine to inhibit PAH in vivo, and assesses the possibility of screening mice according to susceptibility to m-fluorophenylalanine.

PHS-6040
(Rev. 10-76)

Project Description:

The lack of appropriate animal models for the study of enzyme-deficient genetic diseases associated with mental retardation has checked the search for the pathophysiology directly relating the CNS lesion with the enzyme defect. This project was started as a single-laboratory size program to obtain a murin model for the human disorders related to phenylketonuria (PKU). Suspect he erozygote mutant offspring were generated using double-dose X-irradiation of mouse sires (see previous reports, M-NC-8 1972-74). The offspring were screened for the defect in a manner similar to the human screening program, using blood plasma levels of phenylalanine (P), tyrosine (T), and their ratio (P/T) to distinguish abnormal, high values in possible mutants from a distribution of values obtained from mice with normal phenylalanine hydroxylation (PH) systems here shown:



The scheme illustrates the complex nature of the enzyme systems governing the steady-state levels of the amino acids in the blood (two enzymes, a cofactor and a coenzyme). Thus, the screening program, in principle, would detect a deficiency in any component shown or a change in amino acid levels due to oth causes. At the onset of the project the dogma indicated that dihydropteridin reductase (DPR) was in such excess as to permit normal levels of hydroxylatio in the heterozygote mutant deficient in this component. The program included therefore, screening of a large sample of liver biopsies for direct measurements of DPR in the liver. Methods for screening for total dihydrobiopterin in hepatic samples were also used in this project.

The current thrusts of the project are to achieve a pharmacological mode for the suspect PKU mouse in order to obtain the proper criteria for screenin and to utilize the difference in susceptibility to m-fluorophenylalanine (mFP (shown in rats) of normal and PH deficient animals as a possible screening method for PKU heterozygotes. A method for selection of phenylalanine hydrox lation deficient mice by using a drug whose metabolism utilizes the PAH syste to form a highly toxic mitochondrial poison, fluoroacetate, is being studi It had been shown by others that rats treated with parachlorophenylalanine (pCPA) were protected from the lethal effects of high doses of the convulsant drug for 8-11 hours. pCPA is long-known to induce a state of about 95% PH de ficiency in the rat for 24-72 hours after a single injection of about 360 mg/ Attempts to induce a similar state in mice had failed to produce more than 5C inhibition of PAH for short periods, at the same or double that dose. This project applied various modes of administration of pCPA to mice, and measurec

the time course of uptake of the agent into blood plasma and liver, with corresponding in vitro enzyme assays for hepatic PAH. A program for separation of phenylalanine, tyrosine and pCPA from blood plasma and liver extracts at various times after administration of reagent was developed for the automated amino acid analyzer in collaboration with Gladys Deibler, LCM, NIMH. Protein contents of liver samples were determined by colorimetric analysis. Dose response curves for mFPA in the C57BL/6N♂ mouse were obtained by intraperitoneal injection of mice. Comparison of lethality at several levels of PH were made at a mFPA lethality level of about 80%. Various other pretreatments which were expected to interfere with PH were used in attempts to achieve a less than 50% in vitro measurable hepatic PAH level.

Results showed that the difference between the rat and mouse in response to pCPA was due to a difference in uptake and disposal of the agent. Eighteen hours after the administration of 360 mg/kg pCPA in mice scant amount is detected in the blood or liver. The maximum level is reached at 3-6 hours and falls. Doubling the dose leads to approximately twice the maximal concentration in liver and blood from 3-6 hours and falling close to zero by 18 hours. The animals excrete large quantities of phenyl ketones during the 12 hour period. In vitro measurable PAH levels fall during the 6-18 hour period, using the natural cofactor (BH_4) or the synthetic ($DMPH_4$), confirming our previous findings at 24 hours with $DMPH_4$ (see previous report N-MC-8 1974). The inhibition curve was bimodal between zero and 24 hours. The maximal decrease in hepatic PAH activity compared with normal during 0-36 hours was found to be 45-50%. Plasma tyrosine level decreased, phenylalanine increased and the P/T ratio increased 1.5-3 fold during the zero to 9 hour period.

At the 24 hour level of inhibition of PAH and PH, the dose-response for lethality due to mFPA was not shifted; the 50% deficient animals were, therefore, not less susceptible to the agent. A new mode of administration of pCPA was tried with the view towards achieving high levels of pCPA in the blood and liver over longer periods. This was successful. Maximal plasma levels were about 4 fold higher than previously (approaching the levels seen in rats) and persisted over 24 hours. One test of mFPA toxicity at 21 hours (animals were about 70% deficient in in vitro measurable PAH) showed a small shift in the dose-response, indicating some protection.

The results are significant to an understanding of the effects of pCPA in mice and modes of inhibiting PH in vivo in mice. The methods and results will be reported in a joint manuscript with Gladys Diebler, LCM, NIMH. The studies will be continued using C57BL/6J♂ mice because of the frequent illnesses and unreliability of the NIH supplied C57BL/6N mice. Using our route of administration of pCPA we will test for susceptibility to mFPA at several time points with the expectation of finding a given dose of mFPA which would lead to the death of all normals by a certain time after this agent, leaving the "protected" animals alive. These would be treated with phenobarbitol to alleviate the convulsions and lead to survival of the "pharmacological PKU individual".

If the principle of this screening method could be established, it is anticipated that a contract might be drawn up for such a program to be conducted outside of our current limited facilities. We have sufficient knowledge

on how to generate suspect mutants by X-irradiation (or by entrapment of wild mice) at present. An all or none selection based on mFPA toxicity would enable the screening of large numbers of animals at 8 weeks of age.

It should be noted that attempts to further reduce PH in vivo with the administration of methotrexate (MTX)(an inhibitor of DPR) led to potentiation of mFPA. This unpredicted result was studied further. It was shown that low level of DPR activity was found associated with brain mitochondria. Further studies of MTX potentiation of mFPA will be undertaken to increase the statistical impact of the first report.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01081-07 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cerebral Control of Movement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	C. Fromm	Guest Worker	LNP NIMH
	H. Terävainen	Visiting Scientist	LNP NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Neurophysiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.92	1.92	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project involves studies of motor control in subhuman primates, in healthy human subjects, and in patients with parkinsonism and Huntington's disease. Work in primates has shown that rapid ballistic movements depend on central programs operating relatively "open loop," whereas small precisely controlled movements utilize the continuous feedback provided by the "servo" properties of transcortical reflexes. Reflexes of motor cortex neurons are heightened during the servo-mode occurring with small precise movements, while these reflexes are attenuated during the open-loop mode underlying ballistic movement.

Studies in normal human subjects have shown that the response to a kinesthetic stimulus involving muscle stretch consists of: 1) a spinal reflex; 2)a longer latency reflex traversing motor cortex and the pyramidal tract; 3) an even longer latency intended response which involves cerebellum, thalamus, motor cortex, and pyramidal tract. The latency of this third component is in fact kinesthetic reaction time (KRT). KRT may have a minimum value of 70 msec even for a kinesthetic stimulus which reflexly inhibits the muscle which is to respond. KRTs are significantly faster than auditory or visual reaction times. A variety of abnormalities of these responses have been detected in patients with neurological disorders.

Project Description:

Studies are carried out at two broad levels: animal studies of brain processes in monkeys carrying out learned movements, and human studies in normal subjects and patients with psychomotor disorders such as parkinsonism and Huntington's disease.

Animal Studies. For the animal studies the work utilizes two major procedures.

1. Electrical recording. Microelectrodes are used to record the activity of individual neurons during natural, voluntary movement in the monkey. This use of single unit analysis in relation to voluntary movement is analogous to the long-established use of single unit analysis in determining how the brain receives and elaborates its sensory input.

2. Behavioral training. The current work on this project is concerned with the properties of motor cortex neuron discharge in relation to the interaction of central programs and peripheral feedback. For these experiments, monkeys have been trained to make arm movements in order to maintain alignment between two lamps. The paradigm is one of "pursuit tracking." There are two visual displays--one lamp being the target (under the experimenter's control) and the other lamp (under the monkey's manual control) providing a feedback signal to the monkey. In this paradigm, the monkey viewed two horizontal rows of lamps (track and target lamps), each lamp in the track row being directly above a corresponding lamp in the target row. At all times only one lamp in each row was on. Output from a position transducer coupled to the handle selected which of the track lamps was illuminated. While the track display informed the monkey of the actual handle orientation, the location of the target lamp was programmed by the experimenter and indicated how the handle should be positioned. The behavioral sequence established by operant conditioning methods involved:

a) A holding period of continuous correct alignment varying in duration from 1.5 to 2.5 sec. The monkey held the handle vertically to align the central track with the central target lamp.

b) Small error-correcting supination-pronation movements of 1-2^{o} occurred when a misalignment of track and target lamps was caused by the monkey's failure to keep the handle within the narrow hold zone. The same type of small corrective movements could also be triggered by a shift of the track display resulting from an error signal added to the output of the position transducer which controlled the track display.

c) Fast supination-pronation movements of large amplitude (20^{o}) were triggered by a jump of the target to the extreme right or left of the panel. The monkey was rewarded with a drop of juice after the handle had been rapidly rotated in the direction of the target jump; movement time had to be less than 150 msec for reward to be delivered. The monkey's "ballistic" movements were not precisely controlled as to termination, since target zones at both extreme positions were bounded by a physical barrier. These movements commonly reac

velocities which were 20-30 times greater than the peak velocities occurring for the small controlled movements.

d) Immediately after a ballistic movement, the target jumped back to the center and the monkey returned the handle to the central hold zone to initiate a new cycle (step a). This type of large movement was slower than the preceding ballistic movement, becoming progressively slower as the target zone is approached.

e) The handle was coupled to the axle of a DC torque motor which produced a constant torque during steady holding. This torque required either steady supinator or pronator force and resulted in an effective force of 90 gr applied to the monkey's hand. In addition, 50-msec rectangular torque pulses could be delivered to the handle via the motor to study unit sensitivity to peripheral inputs. Randomly alternated pronating and supinating torque pulses were applied i) after 700 msec of steady holding, ii) at a fixed delay of 120 msec after a target jump and thus immediately preceding the ballistic movement, iii) at the start of a ballistic movement, and iv) at the start of a small movement. The latter pulses (iii and iv) were triggered at the time the handle left the central hold zone.

Major Findings:

Motor cortex neuron discharge was examined in relation to movements carried out in the above-described paradigm, with particular attention to large (20^{o}) ballistic movements as compared to small ($\sim 1^{o}$) precisely controlled movements. Among motor cortex neurons related to these pronation-supination movements, about half changed discharge frequency only with the large movement, whereas the other half showed changes both with the large and with the small movement. For many neurons in this latter group, intense discharge was found to occur during even the smallest movements made to achieve accurate positioning of the handle, and such units almost invariably responded (latencies: 20-60 msec) to small handle perturbations which pronated or supinated the forearm. The perturbations, caused by 50-msec torque pulses delivered to the handle, produced handle displacements of about 5^{o}. Typically, the neurons discharging intensely with precise fine movements were reciprocally related (increase vs. decrease of discharge rate) both to the two directions of movement and also to the two oppositely directed torque pulses. On the other hand, neurons active with ballistic supination-pronation movements and inactive with small movements were to a large extent uninfluenced by these kinesthetic stimuli. Activity with small movements and high sensitivity to kinesthetic stimuli inputs was particularly common in pyramidal tract neurons (PTNs) with more slowly conducting axons, and appears analogous to the high sensitivity to segmental inputs in those spinal cord motoneurons first to be recruited in the course of movement (cf. Henneman, E., Principles governing distribution of sensory input to motor neurons, In: The Neurosciences, Third Study Program, F.O. Schmitt and F.G. Worden (eds.), Cambridge: MIT Press, 1974 (pp. 281-291).

For any particular neuron, the responses to torque pulses delivered during postural stability (i.e., accurate holding of the handle) were compared with

the responses to the same torque pulses when delivered immediately before the onset of a ballistic movement, when injected into ongoing ballistic movement, or when applied during precise fine movement. It was found that neuron responsiveness to sensory input is relatively enhanced during accurate positioning and controlled small movement, but reduced before and during ballistic movements. Taken together, these findings demonstrate that the transcortical servo-loop is continuously active and powerful during precisely guided fine movements and posture. The intense and prolonged discharge of precentral neurons during these fine movements must partly reflect the central motor program and partly reflect sensory feedback in a closed loop. The relative contribution of the two inputs to the motor cortex output remains to be fractionated. The relative lack of sensory feedback to motor cortex just before and during a ballistic movement appears to depend on the nature of the central preprogram and on the specific parameters of the movement itself.

Human studies have been aimed at obtaining clearer delineation between the reflex and the intended components of arm movements in normal subjects and patients with psychomotor disorders. To this end we have used a paradigm in which a given direction of intended arm movement is triggered by either of two possible directions of limb displacement, with one direction stretching and reflexly exciting the biceps muscle and the other direction shortening and reflexly inhibiting the biceps musculature which was to become active with the intended response. When the triggering perturbation "unloaded" and inhibited the biceps muscle which the subject intended to activate the intended discha was superimposed on the silence of reflex inhibition and the time of onset of this intended discharge could be accurately identified.

Major Findings:

Using this paradigm, we find that the response to a kinesthetic stimulus involving muscle stretch consists of: 1) a spinal reflex; 2) a longer latency reflex mediated by supraspinal circuits; 3) an intended response which is more rapid than the intended response to a stimulus which does not involve displacement of the responding limb. The supraspinal pathways involved in the second and third components of the response have been elucidated by results of recordings from monkey motor cortex PTNs obtained in a paradigm similar to that employed in the present experiments in human subjects. These recordings revealed two clearly dissociable components of PTN discharge. The first was reflex (being a function of the direction of limb displacement), while the second was intended (being a function of a prior instruction). The reflex component of PTN discharge in monkey begins at latencies of 20-25 msec, and nerve impulses corresponding to the PTN reflex descend to the spinal cord within a few milliseconds, where they give rise to component #2 of the respo observed in human subjects.

The third or intended component of muscle discharge seen in man and monkey depends on volition rather than on reflex processes. This is indicated by results of our studies in monkeys showing that reflex PTN responses beginning at 25 msec can be superseded at 50 msec by responses which depend on the monkey's intention. A similar shift from reflex to volitional control may h

inferred to occur in man. Our recordings from the thalamic and cerebellar structures involved in mediating rapid but volitionally determined responses in monkeys have shown that cerebellar dentate and thalamic ventrolateral nucleus neurons discharge early enough to play a role in rapid volitionally determined motor responses.

Studies in patients with parkinsonism are aimed at seeing how these components of motor response can be used to assess clinical status and aid in the regulation of drug treatment. Systematic studies have been carried out in 20 patients in collaboration with the clinical staff of NINCDS. Results are still too preliminary to serve as a basis for conclusions, but give promise of ultimately providing useful tools for patient assessment and management. Preliminary results have also been obtained in patients with Huntington's disease.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of movement to an understanding of normal and abnormal behavior in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH.

Proposed Course:

Work to extend these studies to patients with psychomotor disorders will continue under a project entitled "Motor Function in Patients with Neuropsychiatric Disorders." For this study the principal diagnostic groups will involve neuropsychiatric diseases in which dementia is a central symptom, such as Huntington's disease, Korsakoff's psychosis, Alzheimer's disease, Gilles de la Tourette's disease, and senile dementia; or behavior disorders where there are strong indications of an organic pathophysiology. Patients with tardive dyskinesia will also be studied, and in addition, patients with parkinsonism and cerebellar disorders will be included in the investigation.

The investigation will involve making precise records of reflex and voluntary arm movements occurring in response to visual, auditory, tactile, and kinesthetic signals. Subjects will grasp a movable handle and maintain it in a certain position while awaiting a stimulus which serves as a signal for them to make an arm movement. Surface electrodes will be applied to the skin overlying the biceps muscle of the arm which is to be used by the subject.

A major new departure in the work involves use of instrumentation which allows intended reactions of the subjects to be triggered by movements of the handle grasped by the subject as well as by the more standard auditory and visual stimuli which are commonly used in studies of reaction time. Furthermore, data collection and computer analysis will allow better estimates of the way in which muscles are activated in the course of movements being carried out by these patients.

Data obtained from the studies in patients will be of value not only in providing a better understanding of the movement disorders which form a part

of the syndromes to be investigated, but also as a means of assessing the effectiveness of drug treatments currently used in patients. Normal subjects will be employed in these studies to provide comparative data for healthy individuals carrying out these tests of psychomotor function.

Publications:

Evarts, E.V.: Neuronal Representation of Acquired Movement Patterns in Primates. In Fentress, J.C. (Ed.): Simpler Networks and Behavior. Sunderland, Mass., Sinaur Associates, 1976, pp. 266-273.

Evarts, E.V.: The Interaction of Central Commands and Peripheral Feedback in Pyramidal Tract Neurons (PTNs) of the Monkey. In Herman, R.M., Grillner, S., Stein, P.S.G., and Stuart, D.G. (Eds.): Neural Control of Locomotion. New York, Plenum, 1976, pp. 808-817.

Tanji, J. and Evarts, E.V.: Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. J. Neurophysiol. 39: 1062-1068, 1976.

Evarts, E.V. and Tanji, J.: Reflex and intended responses in motor cortex pyramidal tract neurons of monkey. J. Neurophysiol. 39: 1069-1080, 1976.

Evarts, E.V. and Granit, R.: Relations of Reflexes and Intended Movemen In Homma, S. (Ed.): Progress in Brain Research. Amsterdam, Elsevier, 1976, vol. 44, pp. 1-14.

Evarts, E.V.: Neurophysiological Mechanisms in Parkinson's Disease. In Birkmayer, W. and Hornykiewicz (Eds.): Advances in Parkinsonism. Basle, Editiones Roche, 1976, pp. 37-54.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 01082-12 LNP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Muscle Function and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. I. Rapoport	Neurophysiologist	LNP NIMH
	V. Nassar-Gentina	Guest Worker	LNP NIMH
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LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.97	.72	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have distinguished fatigue, in response to tetanic stimulation, from post-tetanic potentiation in single muscle fibers of the frog. We have shown that fatigue, as measured by a decline in tetanic tension and the response to short electrical stimuli, is produced when glycogen and ATP concentrations are close to normal and when phosphocreatine is depleted. We conclude that fatigue in these circumstances is due to failure of excitation-contraction coupling, due possibly to accumulation of lactate and H^+ and interference with calcium kinetics, as fatigued muscles can consume residual ATP and produce a contracture when they are exposed to caffeine. Functional failure prior to exhaustion of available high energy metabolites may represent a protective mechanism that prevents death of muscle as well as neuronal cells.

PHS-6040
(Rev. 10-76)

Project Description:

When a whole striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued. Fatigue does not occur in dystrophic muscles and is characteristic of fast but not slow mammalian and amphibian muscle fibers. The complexity of the excitation-contraction process in fast muscles makes it difficult to determine at what stage fatigue sets in. Single muscle fibers of the frog semitendinosus muscle, rather than whole muscles, were selected to study fatigue and recovery. Use of single fibers avoids rate-limited diffusion and the heterogenous fiber population of whole muscle. A fiber was dissected and mounted in a bath of flowing Ringer at 15°C. Tension was recorded by an RCA 5734 transducer tied to one end of the fiber, and was displayed on an oscilloscope screen and a paper chart recorder. Sarcomere length was measured by a laser diffraction technique. The fiber was stimulated by external platinum electrodes to produce twitches or tetanic contractions. At different times during and following a tetanus, it was rapidly frozen in liquid nitrogen and then extracted in perchloric acid at -35°C for determination of protein, ATP, phosphocreatine (PCr) and glycogen by microbiochemical techniques.

Results:

In single muscle fibers, we were able to distinguish the fatigue process following tetanic stimulation from the process of post-tetanic potentiation. Then we correlated, for the first time in fibers, tension with the instantaneous metabolic profile during various stages of fatigue and of recovery from fatigue. We showed that fatigue under the given experimental conditions was directly related to residual PCr concentration, but was not associated with significant depletion of glycogen or of ATP.

Tetanic stimulation at a frequency of 20/sec reduced PCr content of single fibers in proportion to the work done, as measured by the tension-time integral. Tetanic tension fell in relation to the decline of PCr, despite the fact that ATP, the energy source for muscle contraction, was at close to normal level. Caffeine treatment is known to release calcium from internal stores in muscle fibers. Such treatment of fatigued fibers produced maximal contractures and resulted in exhaustion of residual ATP, thus demonstrating that ATP was available for contraction and that fatigue was due to the inability of the fibers to utilize available ATP. On the basis of measurements of lactate and of the ATP/PCr ratio in fatigued fibers, we suggested that H^+ accumulated in them so as to interfere with calcium release in response to excitation or with activation of the contractile mechanism by calcium.

Our findings have been presented in two abstracts: (1) Nassar-Gentina, V., Passonneau, J. V., and Rapoport, S. I.: Fatigue in single muscle fibers of the frog, caused by tetanic stimulation and affected by caffeine, as related to metabolism. Biophysical Journal 17: 173a, 1977; (2) Nassar-Gentina, V., Passonneau, J. V., and Rapoport, S. I.: Relation of muscle fatigue to muscle metabolism. Abstracts 27th International Congress of Physiological Sciences, Paris, France, 1977.

We currently are involved in analyzing our data on the effects of excitation-contraction uncoupling on the relation between tension output and metabolic energy consumption in single fibers. We find that excitation-contraction uncoupling by stretch results in an augmented PCr consumption as related to the excitation-contraction process and not to contraction, whereas such uncoupling by hypertonic treatment reduces PCr in response to excitation.

The relation between contraction, calcium release and uptake, and electrical activity of a single fiber, and kinetic changes in intracellular metabolic and calcium compartments is important not only for understanding the basic mechanisms of excitation-contraction coupling and fatigue and recovery of muscle, but also to see how metabolites are employed generally to support cellular activity. Our finding that fatigue develops despite high levels of ATP corresponds to observations of "power failures" in the stimulated peripheral nerve or sympathetic ganglion. In these tissues, function fails before energy supplies are exhausted. The protection of energy supplies at the expense of function may preserve the life of the neuronal or muscle cell.

Publications:

Vergara, J.L., Rapoport, S.I., and Nassar-Gentina, V.: Fatigue and post-tetanic potentiation in single muscle fibers of the frog. American J. Physiology 232: C185-C190, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01083-11 LNP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transport Mechanisms Across Membranes and the Blood-Brain Barrier

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	K. Ohno	Visiting Fellow	LNP NIMH
OTHER:	M. W. Brightman		LNNS NINCDS
	R. Brady		DMN NINCDS
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	A. Laties		Univ. of Pa.

COOPERATING UNITS (if any)

Laboratory of Neuropathology and Neuroanatomical Sciences and Developmental and Metabolic Neurology Branch, NINCDS; Laboratory of Clinical Science, NIMH; and University of Pennsylvania Medical School

LAB/BRANCH

Laboratory of Neurophysiology

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INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.45	1.7	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to understand the physiological and ultrastructural basis of the blood-brain barrier, and to modify the permeability restriction of the barrier for use in central nervous system pharmacology. We have (a) elaborated the theory and provided morphological and physiological demonstration of osmotic opening of the barrier, (b) refined the technique of osmotic opening so that it can be accomplished without producing brain edema or apparent neurological damage, (c) used osmotic opening to increase barrier permeability to intravascular measles antibody, the enzyme α-mannosidase, ^{125}I-albumin and ^{3}H-norepinephrine, (d) determined pressure threshold for barrier opening by acute carotid artery hypertension, (e) modified the permeability of the blood-aqueous and blood-vitreous barriers of the eye with hypertonic solutions.

PHS-6040
(Rev. 10-76)

Project Description:

The blood-brain barrier at the cerebral vasculature, choroid plexus, and arachnoid membrane covering the brain regulates the ionic environment of the nervous system, has specific mechanisms for the delivery of amino acids and glucose to support brain metabolism, and prevents access of blood-borne proteins to the brain, as well as access of some water soluble drugs (e.g., penicillin and methotrexate).

The aim of this project is (a) to understand the basic morphological and physiological properties underlying barrier function and protecting its integrity; (b) to develop a method to open the barrier that may prove useful in pharmacology of the central nervous system; (c) to understand how the barrier is affected by different stresses to the brain, including the loss of autoregulation of cerebral blood flow; and (d) to understand the blood-ocular barrier and compare it with the blood-brain barrier.

A number of different methods are employed in these studies. Surgical procedures are used to open the skull and dura of rabbit, rat, or rhesus monkey, and to isolate and perfuse the internal carotid artery. Neurological examinations are performed to evaluate function, and methods of perfusion and fixation are used for brain and eye histology. Quantitative chemical techniques are employed for analysis of wet and dry weights of brain tissue, and atomic absorption spectrophotometry employed for studying brain electrolytes. Statistical methods and theory of irreversible thermodynamics are used to consider results quantitatively and to formulate and test models for osmotic barrier opening and the effect of capillary hydrostatic pressure in the brain. Computers are used for data analysis and development of models. Electron microscopy is used to determine passage of horseradish peroxidase from blood to brain, and to evaluate cellular morphology.

The major findings of the project are as follows:

A. Theory and physiological and morphological demonstration of the blood-brain barrier. The blood-brain barrier in the rabbit, rat, or monkey can be osmotically opened in a reversible manner by injecting hypertonic solutions of relatively lipid insoluble nonelectrolytes--e.g., urea, lactamide, or arabinose--into the internal carotid artery for 20 seconds. The barrier to proteins, which is due to tight junctions that connect the cerebrovascular endothelium, is opened by osmotic shrinkage of the endothelial cells and widening of the tight junctions without grossly affecting neuronal function. The theory of osmotic opening, which I had proposed on the basis of previous experiments and extended and confirmed in collaborative efforts by means of electron microscopy, was elaborated in detail and interpreted in terms of general clinical observations (1).

B. Application of osmotic blood-brain barrier opening to central nervous system pharmacology. Osmotic barrier opening provides a means to grant access to the brain of agents normally excluded by the barrier. Before the method

can be applied, however, it is necessary to determine if barrier opening is associated with irreversible changes in the central nervous system. We determined that long-term changes in brain electrolytes or water content did not accompany barrier opening if the procedure was performed without compromising the cerebral circulation (7). Furthermore, monkeys in which the barrier was opened appeared grossly normal neurologically and did not have histological evidence of brain damage. Short-term changes in cerebral metabolism may accompany barrier opening, however. In collaboration with Sokoloff et al., we are showing that cerebral glucose metabolism is increased in perfused brain regions, and we intend to explore the possibility of transient brain edema following opening. An increased glucose metabolism is accompanied by an increased brain uptake of the intravascularly administered glucose tracer analogue, ^{14}C-2-deoxy-D-glucose (4).

We have quantified the extent of barrier opening by employing a number of tracers to study barrier permeability. In the rhesus monkey, carotid infusion of 1.8 to 2 molal arabinose solution significantly elevates brain uptake of circulating neutralizing antibody to measles, and the antibody remains in the brain for up to 4 days after entry (9). In the rat, infusion of 1.8 to 1.6 molal arabinose also can open the barrier to large enzymes, such as human placental α-mannosidase (Barranger, J. A., Pentchev, P. G., Rapoport, S. I., and Brady, R. O.: Augmentation of brain lysosomal enzyme activity following enzyme infusion with concomitant alteration of the blood-brain barrier. Ann. Neurol. 1, 1977 (in press).

We have also shown, using intravascular ^{125}I-albumin and ^{3}H-norepinephrine in the rat, that osmotic barrier opening by 1.8 molal arabinose solution increases brain uptake of these agents two- to fivefold in perfused brain regions. Furthermore, there is a normally significant uptake of ^{3}H-norepinephrine, probably by vascular nerve endings, a fact heretofore unnoticed (Sun, C. L., Chiueh, C. C., Kopin, I. J., Fredericks, W. R., and Rapoport, S. I.: Entry of ^{3}H-norepinephrine and ^{125}I-albumin from blood into brain following osmotic opening of blood-brain barrier in the rat. Abstr. 6th Ann. Meeting, Society for Neuroscience, 2 [Part I]: 506, 1976).

C. Opening of the blood-brain barrier by acute hypertension and by other conditions that modify autoregulation of cerebral blood flow. Autoregulation of cerebral blood flow represents the constancy of flow, caused by changes in cerebrovascular resistance, when systemic blood pressure varies between 50 and 150 mm Hg. I have suggested that there should be a quantitative relation between appearance of brain edema, increased cerebrovascular permeability, and loss of autoregulation (1-3). We currently are developing radiotracer methods to measure blood flow and vascular permeability independently of each other, in order to test this hypothesis. One condition in which autoregulation is overcome is acute hypertension, which we are studying. We have shown in a qualitative fashion that the barrier is opened to intravascular protein when carotid artery pressure is acutely elevated to beyond 170 mm Hg by rapid infusion of isotonic saline into the artery (6). We intend to quantify the effect and determine the mechanism of increased vascular permeability, using electron microscopy.

D. Selective damage of ciliary epithelium of the eye by concentrated solutions, and breakdown of blood-aqueous barrier. We showed that hypertonic solutions infused into the internal carotid, and thence into the ophthalmic artery of the monkey, increase permeability of blood vessels of the retina and optic nerve, and morphologically alter and increase the permeability of the ciliary epithelium (5,8,10). The mechanism of increased vascular permeability probably is like that for cerebral vasculature, as ocular and cerebral vasculature are lined by a continuous layer of endothelial cells that are connected by tight junctions. We have shown that osmotic effects of the ciliary epithelium are irreversible, as compared to their effects at the vasculature. Hypertonic infusion separates the pigmented from the nonpigmented ciliary layer and leads to the destruction of 50 to 80% of pigmented cells. Despite these changes, ocular pressure returns to normal after 1 month, indicating that the nonpigmented layer is critical to aqueous humor secretion.

The results are significant for the following reasons:

1. An understanding of how the barrier regulates the brain environment and controls brain metabolism is important to understanding function and homeostasis of the central nervous system.

2. Osmotic barrier opening is the first method that has been developed that can reversibly allow the entry of normally excluded drugs into the central nervous system, and should be useful as a technique for studying pharmacology of the central nervous system. Entry of antibodies could be used to study measles or other viral growth in the brain, and entry of enzymes could be used to study how the brain cells deal with exogenous enzymes for eventually understanding lysosomal storage diseases. Entry of norepinephrine has already been used to demonstrate central effects of that drug on cerebral blood flow (Pickard et al., Brain Res. 122: 170-176, 1977).

3. We demonstrated that osmotic barrier opening was due to widening and increased permeability of cerebrovascular tight junctions, and suggested that tight junctions also are made more permeable by conditions which alter autoregulation of cerebral blood flow. This hypothesis has not yet been tested but if it is correct, the effects on cerebral function of clinical hypertension, convulsive activity, trauma, and other conditions which alter autoregulation should be reevaluated in terms of the influence of altered barrier function.

4. We have for the first time distinguished between the roles of the pigmented and nonpigmented epithelial layers of the ciliary body of the eye in regulating aqueous secretion. We have shown that the nonpigmented layer alone can control secretion and maintain normal intraocular pressure. Our findings that intraocular pressure can be reduced below normal over a period of months suggest that hypertonic perfusion of ocular tissue might be of eventual use in treating acute glaucoma.

In the next year, we intend to study the short term effects of osmotic barrier opening on brain water and electrolytes and on glucose metabolism. We intend to use the method to allow enzymes into the brain and to study the eventual

disposition of enzymes between intracellular and extracellular brain regions. We shall continue to develop methods, in the conscious rat, to measure cerebrovascular permeability and cerebral blood flow independently of each other, and to apply these methods to conditions, such as hypertension, and to states, such as aging, to determine blood flow and permeability and their correlated changes when autoregulation of flow is lost or overcome.

Publications:

1. Rapoport, S.I.: Blood-Brain Barrier in Physiology and Medicine. New York, Raven Press, 1976, 316 pp.

2. Rapoport, S.I.: Blood-brain barrier permeability, autoregulation of cerebral blood flow and brain edema. In McLaurin, R.L. (Ed.): Head Injuries. New York, Grune & Stratton, 1976, pp. 115-121.

3. Rapoport, S.I.: Modification of cerebrovascular permeability by hypertonic solutions and conditions which alter autoregulation of cerebral blood flow. In Cervos-Navarro, J., Betz, E., Matakas, F., and Wüllenweber, R. (Eds.): Erwin Riesch Symposium on the Cerebral Vessel Wall. New York, Raven Press, 1976, pp. 215-223.

4. Spatz, M., Rap, Z.M., Rapoport, S.I., and Klatzo, I.: Effects of hypertonic solutions and of mercuric chloride on the uptake of [^{14}C] glucose analogues by rabbit brain. Neuropathology Applied Neurobiology 2: 53-61, 1976.

5. Laties, A.M. and Rapoport, S.I.: The blood-ocular barriers under osmotic stress: studies on the freeze-dried eye. Archives of Ophthalmology 94: 1086-1091, 1976.

6. Rapoport, S.I.: Opening of the blood-brain barrier by acute hypertension. Experimental Neurology 52: 467-479, 1976.

7. Rapoport, S.I., Matthews, K., and Thompson, H.K.: Absence of brain edema after reversible osmotic opening of the blood-brain barrier. In Pappius, H.M. and Feindl, W. (Eds.): Dynamics of Brain Edema. Berlin, Springer-Verlag, 1977, pp. 18-22.

8. Okisaka, S., Kuwabara, T., and Rapoport, S.I.: Effect of hyperosmotic agents on the ciliary epithelium and trabecular meshwork. Investigative Ophthalmology 15: 617-625, 1976.

9. Hicks, J.T., Albrecht, P., and Rapoport, S.I.: Entry of neutralizing antibody to measles into brain and cerebrospinal fluid of immunized monkeys, after osmotic opening of the blood-brain barrier. Experimental Neurology 53: 768-779, 1976.

TYPING GUIDE SHEET

10. Laties, A., Armaly, M., Rao, R., and Rapoport, S.: The blood-ocular barriers under stress. In Freeman, H.M., Hirose, T., and Schepens, C.L. (Eds.): Vitreous Surgery and Advances in Fundus Diagnosis and Treatment. New York, Appleton, Century & Crofts, 1976, pp. 559-568.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 01084-06 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Miles	Senior Staff Fellow	LNP NIMH
OTHER:	D. J. Braitman	Guest Worker	LNP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.7	2.0	2.7

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to examine the neural basis of the adaptive mechanisms responsible for maintaining appropriate performance levels in the vestibulo-ocular reflex. Our present concern is to determine exactly where the modifiable elements are located through lesions and single unit recordings in the vestibulo-ocular reflex pathways.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Experimental objectives were twofold: 1. Behavioral studies have attempted to define quantitatively the dynamic characteristics of the vestibulo-ocular reflex (VOR) after it has been modified through prolonged optical reversal, magnification or reduction of the visual input normally associated with head movements. 2. Chronic single unit recording techniques were initiated with the object of locating the site of the neural modifications which underlie these adaptive changes in the VOR.

Methods: In the behavioral projects, we examined the effects of manipulating the visual input normally associated with head movements, on the VOR. Normally, monkeys, like men, can rely on the VOR to maintain a stationary retinal image during head turns. However, our previous studies have shown that this system is modifiable and subject to some visually mediated optimization process which assures that the operation of the VOR maintains maximum stability of the retinal image during head turns; thus, when animals wear telescopic spectacles, the VOR adapts to the new conditions by increasing or decreasing its gain depending on the power of the lenses being worn by the animal. In the present experiments we have used precise quantitative methods to examine the exact time course of these changes, their reproducibility in both the same and different animals, and the stability of such changes in the absence of sustaining visual feedback (for example, when the head is immobilized for a prolonged period). Additional experiments concerned the effects on the VOR of prolonged optical reversal of vision, produced by dove prism spectacles.

Single unit recordings were made in the vestibular nerve and the flocculus of a) normal monkeys, and b) monkeys whose VOR has been modified by prolonged exposure to dove prism or telescopic spectacles. Monkeys were seated in a special chair which could be oscillated about the vertical axis by a servo-controlled torque motor. Since the animals' heads were secured to the chair, the chair rotation provided controlled vestibular stimulation. The vestibular sensitivity and phase of individual neuronal responses was calculated from the discharge modulations accompanying controlled sinusoidal oscillations of the chair at varying frequencies. Our previous experiments have shown that the discharge of semicircular canal primary afferents is not immediately influenced by ongoing visuo-vestibular experience but the concern of the present experiments was to look for long-term influences. For the recordings in the flocculus, however, it is necessary to use monkeys trained on a fixation task since this area carries oculomotor as well as vestibular signals; special paradigms were devised to dissociate these normally co-varying signals and permit quantification of each separately. Data were recorded on analog magnet tape and subsequently digitized for quantitative analysis on a PDP-12 comput

Major Findings:

1. Animals that have worn telescopic spectacles show adaptive changes in the gain of their VOR. The normal rhesus monkey has a VOR gain of close to unity, and after x2.0 lenses values up to 1.85 are recorded, while after x0.5 lenses values as low as 0.6 are found. These changes take 2 to 5 days to reach

asymptote, and recovery following removal of the spectacles takes a similar period of time. These changes follow an exponential time course so that the rate of change of gain is proportional to the error, i.e., the difference between the actual VOR and that called for by the power of the lenses. There is no "progressive learning effect," i.e., with subsequent repetitions of the spectacle experience, the time required to adapt does not shorten. Prolonged head immobilization after prior adaptation considerably delays recovery back to normal. The compensatory eye movements following these various exposures are always of very similar gain for both self-generated and passively imposed head movements. In addition, gain changes can be brought about by spectacle experience even when all of the head movements were passively imposed, though the recalibration under these circumstances seems to be more complete. Monkeys that have worn dove prism spectacles, producing left-right reversal of vision, show a marked reduction in their horizontal VOR, which falls to about one-third normal over a 9-day period but then shows no further changes (the experiment was continued for a further 2 months). Only minor phase changes (lag) were observed. However, when subjected to daily sessions of forced oscillation about the vertical axis, gradual reversal of the horizontal vestibulo-ocular responses was seen to develop, though very slowly (a period of several weeks). These "reversed" responses have unusual dynamic characteristics, often give way to normal directed responses under some conditions, and are of very limited functional significance: 1) With lower frequency sinusoids, eye velocity became markedly phase advanced on head velocity, and with alternating velocity steps (triangle waveform oscillations) the "reversed" responses showed roughly exponential decays with time constants of 0.6 to 1 second. This contrasts with time constants in excess of 20 seconds in both normal monkeys and those that have undergone "pure" VOR gain changes as a result of wearing telescopic spectacles. 2) Caloric nystagmus induced by cold water irrigation frequently commenced in the "reversed" direction but rapidly reverted to the normal after only a second or two, eventually achieving peak slow wave velocities about one-third normal. These brief "reversed" responses were at best very weak and easily overlooked, but since they were never observed in the normal animal, they are unlikely to be due to some spurious thermal transient in the irrigation fluid. 3) Self-generated head turns in total darkness (measured by coupling the head to a rotary potentiometer) were usually accompanied by "reversed" compensatory eye movements; however, even with the largest head movements (up to 100°) these "reversed" eye movements were invariably small (rarely more than 6°) and always transient, terminating within .3 to .6 seconds even if the head movement was still in progress. Indeed, when a head movement did continue beyond this time, a second period of ocular "compensation" was often evident in which the eyes now moved, albeit weakly, in the normal direction (i.e., opposite to the head), so that the overall ocular response was biphasic.

2. Single unit data have been gathered from the vestibular nerve and flocculus of three normal monkeys and four gain-changed animals, and recordings are still underway. So far, data from the three normals and one of the gain-changed animals have been computer analyzed. The preliminary indications are that the modifications underlying the adaptive capability of the VOR do not occur in either the labyrinth or the flocculus. Indeed, such changes as are seen

in the flocculus suggest rather that this structure receives a signal which has already been partly modified.

Significance to Biomedical Research and the Program of the Institute:

1. The effects of telescopic spectacle experience on the gain of the compensatory eye movements coupled to head turns clearly indicate that the primate vestibulo-ocular system is both plastic and adaptive seeking always to optimize retinal image stability. The relative simplicity of this system, its easy quantification, and our ability to induce modifications in its performance without apparent pathological side effects, make it a powerful new substrate for studying plastic mechanisms in brain function. Prolonged optical reversal of vision results in both gain and phase changes in the VOR, but the dynamics of the "reversed" responses are very abnormal suggesting that they are subserved by a different neural substrate from that mediating pure gain changes. Thus there seems to be more than one modifiable element in this system.

2. The single unit recordings are being undertaken to establish the exact locus of the modifiable elements in the VOR. A great deal is known about the anatomy and physiology of the VOR, which represents one of the best examples of adaptive plasticity currently available in the primate brain. Once we know exactly where the modifiable elements are located, a whole new area of research related to aspects of motor learning will be opened up.

Proposed Course:

Studies will continue to search for the locus of the modifiable elements in the VOR. The behavioral studies will examine the adaptive capability of monkeys subjected to plugging of the semicircular canals on one side. Older studies on canal plugging in cats suggest that the canals on both sides must be operative for adaptive changes to occur and we propose to examine this in monkeys using more extensive quantitative methods. A positive result in these experiments would suggest an important adaptive role for the commissural connections in the system. The single unit studies will continue to pursue this problem stage by stage, starting at the periphery and working in. If changes are not seen in the primary afferents or the flocculus, which seems likely from our preliminary experiments, then we shall pursue the problem to the next stage, the vestibular nuclei.

Publications:

Miles, F.A.: The Primate Flocculus and Eye-Head Coordination. In Brooks, B. and Bajandas, F. (Eds.): Eye Movements: ARVO Symposium. New York, Plenum Press, 1977, pp. 75-92.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01085-04 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolic Correlates of Brain Functional Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Schwartz	M. O. (Research)	LNP NIMH
OTHER:	H. Gainer	Chief, Section on Functional Neurochem.	BB NICHHD

COOPERATING UNITS (if any)
Section on Functional Neurochemistry, Behavioral Biology Branch, NICHHD

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Laboratory of Neurophysiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.12	.37	.75

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to demonstrate regional metabolic correlates of brain functional activity using the [^{14}C] 2-deoxy-D-glucose (2-DG) autoradiographic method which reflects brain glucose utilization. Present projects include (A) the construction of an atlas which compares 2-DG autoradiographs from injected rat brains with corresponding labeled Nissl sections, and (B) use of 2-DG as a functional marker for the activity of the suprachiasmatic nucleus.

PHS-6040
(Rev. 10-76)

Project Description:

This project seeks to show that the study of regional brain metabolism can provide a mirror for alterations in brain functional activity. The method used in these studies involves mapping of brain glucose consumption. As the brain under normal circumstances is dependent on the combustion of glucose for its energy, this method allows us to functionally map those brain regions which change their metabolic activity in association with specific physiological and/or experimental conditions.

Glucose consumption is measured using a technique developed by Sokoloff and his associates in the Laboratory of Cerebral Metabolism, NIMH, which allows determination of the rates of glucose consumption of individual structures within the central nervous system by utilizing tracer amounts of [^{14}C] 2-deoxy-D-glucose (2-DG). A tracer dose of the radiochemical is injected intravenously in an experimental animal, the animal sacrificed after 45 minutes, the brain frozen, and frozen sections are cut and autoradiographed on X-ray film. The autoradiographs can be used to compare the relative rates of glucose consumption of brain structures.

Recent studies using this technique in the Laboratory of Cerebral Metabolism and the Laboratory of Neurophysiology have mapped alterations of regional glucose uptake associated with alterations of functional activity. Regional, activity-related increases in glucose consumption occur in mammalian central nervous system during sciatic nerve, vestibular, and olfactory stimulation, during penicillin-induced seizures and periods of swimming. Decreases in glucose consumption follow unilateral eye enucleation and unilateral occlusion of an external auditory canal.

Experiments Include:

A. 2-DG Autoradiographic Maps in Resting, Awake Rats

Interpretation of the functional studies described above requires a knowledge of both the pattern of regional brain glucose consumption in the normal animal and the anatomic identities of the neural structures of interest, structures which on autoradiographs are identifiable only by regional variations of optical density. This project provided a framework for the interpretation of further functional studies in the rat by describing (1) the pattern of regional brain glucose consumption in the normal, resting, awake rat, and (2) the anatomical identities of the autoradiographically visualized brain structures.

B. Use of 2-DG Uptake as a Functional Marker in the Suprachiasmatic Nucleus

A number of studies have suggested that there exists a central rhythm generator (an endogenous neural clock) for generating and regulating circadian rhythms, and on the basis of lesion studies the suprachiasmatic nuclei (SCN) have been hypothesized as one possible locus for such a clock. In this project, in collaboration with Dr. H. Gainer, NICHHD, a directly measurable

property of the SCN, glucose consumption measured by the 2-DG autoradiographic method, was examined to determine if it would be affected by environmental light and exhibit circadian rhythmicity in intact (unlesioned) animals. It was found that the SCN glucose consumption could be "turned on" by exposure of the animal to light and that there was some evidence compatible with an endogenous rhythm of SCN glucose consumption even in the absence of environmental light. More experiments to prove the existence of an endogenous circadian rhythm of SCN glucose consumption and to determine what biochemical event(s) account for the circadian rhythm of SCN glucose demand are still necessary.

Principal investigator has left the Laboratory of Neurophysiology and the project is terminated.

Publications:

Schwartz, W.J. and Sharp, F.R.: Autoradiographic maps of regional brain glucose consumption in resting, awake rats using [^{14}C] 2-deoxyglucose. J. Comp. Neurol. (in press).

Schwartz, W.J. and Gainer, H.: Suprachiasmatic nucleus: use of [^{14}C] 2-deoxy-D-glucose uptake as a functional marker. Science (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 01088-02 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Functional Studies of the Substantia Nigra

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. J. Schwartz M. O. (Research) LNP NIMH

COOPERATING UNITS (if any)

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.37	.37	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to clarify both the normal function and the pathophysiology of the substantia nigra. Present experiments include using the [^{14}C] 2-deoxy-D-glucose (2-DG) autoradiographic method to study the patterns of altered forebrain glucose consumption which result from (A) non-specific and specific (6-hydroxydopamine) lesions of ascending dopaminergic pathways and (B) pharmacological treatment of lesioned animals with intravenous apomorphine.

PHS-6040
(Rev. 10-76)

Project Description:

This project aims to understand both the normal function and the pathophysiology of the substantia nigra by using the [^{14}C] 2-deoxy-D-glucose (2-DG) tracer technique for measuring brain glucose consumption (see Project No. Z01 MH 01085-04 LNP). The substantia nigra is of research interest for a number of reasons. As part of the basal ganglia anatomically, its role in brain function remains quite unclear. The cell bodies of the substantia nigra contain dopamine, and their axons form a well-described dopaminergic nigrostriatal tract. Clinical interest in this system has been generated by the observation that decreased dopamine in the striatum and substantia nigra has been correlated with the presence of rigidity and akinesia in Parkinson's disease, and that basal ganglia degeneration may be an important factor in the pathogenesis of this disorder. Finally, a wide variety of brain functions has been attributed to dopamine, including regulation of feeding, reward mechanisms, and in the pathogenesis of schizophrenia.

To initially characterize the pattern of altered brain glucose consumption which might result from destruction of ascending dopaminergic pathways, large unilateral electrolytic lesions of the lateral hypothalamic area in the region of the ascending pathways were made. The brains of these animals revealed that the glucose consumption of a number of structures rostral and ipsilateral to the lesion was depressed compared with that of the contralateral side. The most significant effects were found in sulcal prefrontal cortex, frontal cortex, parietal cortex, and caudato-putamen; but not all forebrain structures were affected, and no structure caudal to the lesion was involved. The right-left metabolic asymmetry was present as early as 2 hours postoperatively and persisted virtually unchanged for at least 14 days. Finally, study of one other biochemical pathway, the incorporation of intravenously injected [^{14}C] L-leucine into its formalin-fixed metabolic derivatives (mostly proteins), in the lesioned animals revealed that there was no lesion-induced left-right disruption of this pathway. Since at least this metabolic pathway appeared preserved in lesioned animals, the observed lesion-induced disruption of forebrain glucose consumption was probably not a consequence of inert ipsilateral brain tissue. Furthermore, the disruption of the metabolic pathways for forebrain glucose consumption may have been a specific effect of the lesions and not merely a non-specific reflection of a lesion-induced alteration of whole brain mechanism.

To determine what role ascending dopaminergic pathways might play in the development of the metabolic sequelae of the lateral hypothalamic lesions, 6-hydroxydopamine was unilaterally injected into the substantia nigra. The asymmetry of glucose consumption could be partially reproduced in some of the forebrain structures, most significantly in the caudato-putamen, by such lesions. Destruction of the cell bodies which give rise to the ascending dopaminergic nigrostriatal bundle is one consequence of 6-hydroxydopamine lesions of the substantia nigra, so it is possible that the destruction of this bundle by the lateral hypothalamic lesions was partially responsible for the disrupted pattern of caudato-putamen glucose consumption observed after lateral hypothalamic lesions.

As one test of this hypothesis, apomorphine, a dopamine receptor agonist, was administered to animals with lateral hypothalamic lesions to determine whether pharmacological stimulation of denervated striatal dopaminergic receptors could correct the metabolic deficit in the caudato-putamen. When apomorphine was given to the lesioned animals 14 days postoperatively, the glucose consumption in sulcal prefrontal cortex, frontal cortex, and parietal cortex remained ipsilaterally depressed, but the right-left asymmetry of the glucose consumption in caudato-putamen had disappeared (and had even reversed in anterior caudato-putamen). Interestingly, when apomorphine was given to the lesioned animals 3 days postoperatively, there was no effect on the metabolic asymmetry of any of the forebrain structures, including that of the caudato-putamen.

Therefore, the observed asymmetry of glucose consumption in caudato-putamen after unilateral electrolytic lesions of the lateral hypothalamus can be (1) partially reproduced by 6-hydroxydopamine injections into the substantia nigra, and (2) reversed by intravenous apomorphine. The observation that a 1 mg/kg dose of apomorphine restores caudato-putamen glucose consumption 14 days after lesions had been made but is ineffective 3 days postoperatively may possibly be explained by a leftward shift in apomorphine's dose-response curve as a result of the gradual onset of "denervation supersensitivity" of the post-synaptic dopaminergic receptors in the caudato-putamen.

The causal association between the metabolic effects of surgical and pharmacological manipulations of the dopaminergic nigrostriatal bundle and the behavioral effects of such manipulations remains to be elaborated. Similarly, the patterns of altered brain glucose consumption which might result from lesions of other intrinsic, diffusely projecting brain pathways are the subjects of future research.

Principal investigator has left the Laboratory of Neurophysiology and this project is terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01089-01 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cortical Response to Sensory Input as a Function of Steady State Motor Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Wolpaw	Staff Fellow	LNP NIMH
OTHER:	T. R. Colburn	Chief, Section on Technical Development	IRP NIMH

COOPERATING UNITS (if any)

Section on Technical Development, IRP, NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.85	1.0	1.85

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is a study of the responses of single units in cortical areas 4, 3a, and 3b of macaca mulatta to wrist flexor and extensor torque pulses or isolated muscle stretch superimposed on a range of steady torques. The aim is to determine the relationships between cortical response to torque pulses or isolated muscle stretch and steady state torque and the relationships between cortical response to torque pulses or isolated muscle stretch and unit behavior during steady state torque. In connection with this project we have developed a technique which permits stretch of individual muscles in the awake behaving primate by direct application of calibrated force unaccompanied by other stimuli. An external electromagnet applied force to a small piece of permeable metal chronically imbedded in the musculotendinous junction. We are using it as an aid in the identification and characterization of units in cortical area 3a, the region to which primary spindle afferents project.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The sensory-motor cortex of man and subhuman primates may be conceived of as a part of a servo-system, generating outputs leading to movement and in turn receiving sensory feedback resulting from movement. The feedback has a major role in determining subsequent cortical output. Thus understanding of how cortical neurons participate in motor control requires understanding of how cortical response to sensory feedback is related to the movement underway. The objective of this project is to test the hypothesis that the signs and strengths of the feedback signals returning to neurons in sensory motor cortex are systematically related to the signs and strengths of the output signals generated by these neurons in the course of movement performance. In the experiment transient feedback signals are presented to a monkey while the steady state motor activity required of the monkey is varied. Delineation of the relationships between neuronal responses to these transient feedback signals and the concurrent steady state motor output is the goal of the study.

Methods:

The monkey is rewarded for maintaining a straight wrist posture, neither flexed nor extended. A torque motor generates forces which oppose the efforts of the monkey. The direction and magnitude of the force generated by the torque mot thus dictates the direction and magnitude of the opposing force which must be generated by the monkey. The monkey is rewarded for exerting a force equal and opposite to the force generated by the motor. The force generated by the torque motor may be varied over a large number of levels ranging from a level requiring strong wrist extension by the monkey to one requiring strong wrist flexion by the monkey. Two types of transient feedback stimuli may be delivered: flexor or extensor 50 msec torque pulses generated by the torque motor, and 100 msec isolated muscle stretches. To allow the isolated muscle stretch, a technique has been developed involving implantation of a small piece of metal into the musculotendinous junction and application of force to this implant and thus to the muscle via an external electromagnet.

Following training of the monkey, single unit activity is studied in sensory motor cortical areas 4, 3a, and 3b. Unit activity, handle position, and stimulus codes are recorded and analyzed off-line by a PDP-12 computer. For each unit, the analysis focuses on the frequency and pattern of discharge at each steady state force level, the change in frequency and pattern of discharge produced by torque pulses or muscle stretch at each steady state force level and the relationships between torque pulse and muscle stretch responses and behavior at the steady state force levels.

The brain is marked grossly at the time of death; gross and microscopic examination is performed to determine the state of the cortex and to correlate penetrations with areas of cortex.

Findings:

Data have been obtained from 1,000 units in two monkeys. The primary interest is in those units which respond within 50 msec, that is, during the torque pulse and prior to change in the direction of handle movement. Formal analysis of the data awaits histological determination of the types of cortex from which each unit was recorded. Preliminary analysis of data from the first animal suggests that for areas 3a and 3b, those units whose response to a torque pulse of given polarity varies with the steady state torque usually give a greater response when the steady state torque is of the same polarity, and those units whose steady state activity is greater with a steady state torque of a given polarity usually give a greater torque pulse response with steady state torque of the same polarity. These data appear to suggest a positive correlation between the magnitude and polarity of steady state motor output and the magnitude and polarity of cortical unit response to sensory input.

Significance:

Further elucidation of how relevant sensory feedback is utilized in ongoing performance of skilled motor activity is essential to better understanding of motor control. Some understanding of this problem has been achieved on the spinal cord level in the relationships described between alpha motoneuron activity and gamma motoneuron activity and muscle spindle responsiveness. This project is an attempt to begin delineation of analogous relationships on the cortical level, to determine how the cortical response to relevant sensory input during motor behavior is related to that behavior.

Proposed Course:

Following completion of recording in the second animal, full analysis and categorization of unit behavior and correlation of behavior with cortical areas will be carried out, and the conclusions indicated by the data in regard to the relationships between cortical response to sensory feedback and ongoing motor output will be delineated. In addition, development of the electromagnetic muscle stretch technique will be continued with further investigation of the design of the electromagnet, current supply and control, implant form and magnetic properties, and surgical techniques.

TYPING GUIDE SHEET

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01090-01 LNP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

CNS Connections Studied by Amino Acid Autoradiography and Horseradish Peroxidase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Miles Herkenham Staff Fellow LNP NIMH

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2.45	.75	1.7

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A neuroanatomical research laboratory has been equipped for the newly developed fiber tracing techniques of tritiated amino acid autoradiography and horseradish peroxidase (HRP) histochemistry. Injections of the tracer chemicals into discrete regions of the rat brain are made by iontophoresis. The autoradiographic material is intended to show the efferent projections of various midline and intralaminar thalamic cell groups over widespread regions of the cerebral cortex. Special attention will be paid to the laminar distribution of thalamic afferents in the cortex. The HRP material can provide details about the sources of the cortical projections. Taken together, these data may provide structural evidence for mechanisms of thalamocortical integration that have not previously been appreciated.

PHS-6040

Objectives:

This project uses the most recently developed neuroanatomical tracing techniques in order to map connections within the brain. Work in progress has dealt with the cortical projections of the intralaminar, midline, and certain paramedian thalamic nuclei in the rat. Work to be undertaken will focus on interconnections between cerebral structures in the monkey. The cell groups which have been studied in the rat became of particular interest over 30 years ago when researchers found that electrical stimulation of sites lying within the domain of the so-called "nonspecific" thalamus could elicit a subtle yet widespread evoked cortical response. The electrophysiological data further suggested a topographic relationship between sites of stimulation within the midline and intralaminar cell groups and foci of maximal cortical evoked response. Unfortunately, however, the traditional neuroanatomical tract-tracing techniques, based on the lesion method, could find only minimal evidence for a nonspecific thalamocortical connection with a widespread areal distribution. Consequently, the early workers abandoned their interest in the possibility that the nonspecific thalamic system may underlie some basic phenomena related to levels of cortical and behavioral arousal, and even perhaps conscious integration. The present studies aim to re-kindle the interest by providing the long-sought anatomical bases for the electrophysiological phenomena.

Methods Employed:

The techniques of anterograde fiber tracing by tritiated amino acid autoradiography and location of cells of origin by retrograde axoplasmic transport of HRP, both developed in the last decade, have provided more sensitive and artifact-free data than the older degeneration methods. Moreover, the deposition of the tracer enzymes in the brain by microiontophoresis produces injection foci that are much smaller, yet more concentrated, than those produced by hydraulic pressure. The combination of these newly developed methods has made possible the aforementioned research.

Since the present project was undertaken in LNP in January 1977, extensive progress has been made toward the creation of the necessary facilities for autoradiography, HRP histochemistry, and related histology. Glass stills for distilled water production, laboratory apparatus and glassware, chemicals and stains, precise stereotaxic surgical facilities, microscopes and photomicrography equipment have been acquired and made operative. Technical personnel are being trained to do exacting ultra-clean histology required for producing good autoradiographic material. A humidity-controlled darkroom, soon to be installed, will be used for dipping slides containing sectioned and mounted brain tissue into radioactive-sensitive emulsion and subsequent drying of the slides.

Major Findings:

Previous work by the principal investigator utilizing the techniques described above has shown a remarkable and unique projection to the outer layer of almost the entire neocortex originating from the nucleus ventralis medialis thalami in the rat. Another widespread and hitherto unrecognized projection to the

hippocampus and limbic cortices was found after placing a mixture of ^{3}H-leucine and ^{3}H-proline into the nucleus reuniens of the thalamic midline group.

Preliminary results from cases of injection of intralaminar nuclei suggest another instance of widespread neocortical distribution, but in contrast to that of the nucleus ventralis medialis, intralaminar projections appear to terminate in deep cortical layers. Some material suggests that thalamic inputs may be distributed two or even three layers, and current experiments must determine whether the multiple and spaced laminations arise from a single or adjacent thalamic cell groups. Further analyses must also provide the topography of the thalamocortical and thalamostriatal projections. Much of this information can be acquired from HRP case material.

The surgical and histological facilities for HRP histochemistry were operative in April 1977 and we have since begun investigations aimed at providing details of the aforementioned thalamic projections as well as some impressions about the sources of inputs to the thalamic nuclei under investigation. For example, we have found that a major and topographic input to the nucleus ventralis medialis arises from the substantia nigra pars reticulata. From such results it becomes possible to better understand the mechanisms of nonspecific thalamic impulse transmission.

The HRP histochemical procedures for tracing neural connections in the retrograde direction are undergoing constant revision and improvement in many laboratories. A secondary purpose to the present HRP studies is to develop the most sensitive and reliable histochemical techniques. Factors considered are the quality and brand of HRP, the type of chromogen used to produce the dark HRP reaction product, and various procedures that may enhance the uptake of HRP by axon terminals. The results of experiments done thus far show in this regard that iontophoretically deposited HRP labels cells in afferent regions so clearly that other factors do not significantly affect the sensitivity of the method.

Finally, a completely different approach to a better understanding of thalamic function is the study of regional glucose metabolism measured by brain consumption of radioactive 2-deoxy-D-glucose. The laboratory is equipped for this procedure and a more detailed description of the method appears in another report (see Project No. Z01 MH 01085-04 LNP). Relative changes in glucose consumption of thalamic nuclei have resulted from various anesthetics given intraperitoneally to rats. Future work will further analyze the specific effects.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

These data, when complete, will show more precisely the nature of thalamic termination in the cerebral cortex. The newly developed neuroanatomical histochemical tracing methods, wherever applied, tend to reveal CNS connections in more detail and entirety than was previously demonstrated or imagined. In this case, they may show that a traditional concept--that thalamocortical fibers terminate mainly in the intermediate layers--must be revised in light of the demonstration that the cell groups lying outside the main thalamic relay nuclei

have unique forms of laminar afferentation. Just as the "wiring plan" of the cerebral cortex must include the lamination of specialized cortical efferent cells, now the various types of laminar inputs must be included for a better understanding of cortical function.

Likewise, the great spread of these nonspecific thalamic projection systems over many functionally distinct cortical regions suggests an important form of neural integration. The emerging possibility that the widespread projections arise from thalamic nuclei which are themselves innervated by components of the motor system (substantia nigra, cerebellum, corpus striatum) suggests some role of movement in states of awareness and arousal. The demonstration of similar neural connections in higher mammals would be useful.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01335-07 SMRC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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7.2	3.2	4.0

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XX (a) HUMAN SUBJECTS XX (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project summarizes the extensive studies with chronic schizophrenics including the development of a rating scale for measuring change over time the therapeutic testing of 5-hydroxytryptophan, tryptophan, alpha-methyl-para-tyrosine, pineal extract, physostigmine, lithium, baclofen, thyrotropin releasing hormone, naloxene, as well as many biochemical determinations including monoamine oxidase.

Project Description:

Objectives:

To test existing and create new hypotheses relating to the possible organic basis of schizophrenia. This project attempts to synthesize psychological, physiological, and biochemical disciplines around the subject of schizophrenia.

Methods Employed:

All studies have been carried out in phenothiazine refractory chronic schizophrenics. The drugs were given and measurements made in a double-blind fashion.

Major Findings:

I. The Rating Scale

Experience gained with the rating scale we had been using on the research wards to record clinical status indicated that it had many shortcomings. We, therefore, undertook to design a more useful nursing scale that could yield quantitative estimates of the level of psychopathology of chronic schizophrenic To be useful, the scale had to be objective as possible and be comprehensible to nursing staff trained to the level of nursing assistant.

Numerous conferences were held with the nursing staff to determine which psychiatric concepts were comprehended and could be inquired about on a rating form. Sample forms were devised and tested in the following manner: Every staff member on each shift would complete the rating on two patients. The results of this exercise were then subjected to statistical analysis in order to determine reliability and to find if, in fact, any meaningful consensus had been found as to the meaning of the questions. Different pairs of patients were used and the same patients were tested again to determine if reliability was consistent over time.

We have evolved a rating scale that appears usable by nursing staff trained at the nursing assistant level which will accurately reflect the daily clinical status of the patient. The new nurses' rating scale was tested extensively and various ambiguities eliminated. In an effort to shorten the scale correlations were run between specific questions, these correlations did not hold consistently in each patient. Thus, no questions could be demonstrated to be expendable. Computor programs for processing the scale have been devised and a new optical scanning machine activated so that quick feedback can be gained from each patient.

The Inpatient Behavioral Rating Scale (IBRS) is an inventory designed to record daily nursing observations of mood and behavior of patients in a clinical setting. It consists of 26 items characterizing a broad range of psychopathology and is particularly suitable for use where change in mood and be-

havior is relevant to clinical investigation. It is formated on an optical scan sheet for ease of computer entry and analysis. In over a year of use on two clinical research wards, the IBRS has been found discriminating, reliable, and easy to use by the nursing staff.

We have also contributed materially to the development of a new nurses' rating scale proposed for use throughout the Bethesda and Saint Elizabeths' wards of NIMH. This scale has been keyed particularly to the well established BPRS of Overall and Gorham, and if it can be successfully taught and completed by nursing personnel, will considerably enhance the process of communication of our findings to the psychiatric professional community. This effort is part of a larger effort at standardizing to some extent the procedures and forms used on the clinical units in Bethesda and at Saint Elizabeths Hospital.

II. Serotonin and Its Precursors

A. Behavioral: The study has now been underway for about five years. The first three patients were given alpha-methyl-5-hydroxytrytophan for one month preceded and followed by one month placebo periods. The active drug made these patients worse.

The subsequent 18 patients were given L-5-hydroxytryptophan together with a peripheral decarboxylase inhibitor (MK-486). MK-486 blocks the conversion of 5HTP to serotonin outside the brain and because MK-486 does not enter the brain makes more 5HTP available for conversion to serotonin within the brain parenchyma. In addition, it is thought that MK-486 reduces 5HTP's side effects.

The patients were given placebo for one month, the active drugs for up to three months, and then a second placebo for one month. Behavioral ratings were done by nurses four times daily. The last 7 days of the highest active drug period were compared to the last 7 days of both placebo periods. For a change to have taken place, we required that the active drug period had to be higher or lower than both placebo periods ($p<0.05$, 2 tailed "t" test). By the criteria indicated, 3 patients have improved, 3 have become worse, and for 12, there was no change. It must be emphasized that these comparisons were made against placebo only and as yet, we have not determined how 5HTP compares to conventional drugs. What is clear is that these drug combinations chemically differentiate at least two kinds of schizophrenic patients; those who become better and those that become worse. Further efforts to determine if these chemical differentiations correlate with any behavioral distinctions are being made.

In order to determine if there are any additive effects of 5HTP and neuroleptics, we are now treating our patients with a constant dose of a neuroleptic, MK-486 and 5HTP (3-6 mg/day). The first three patients were given chlorpromazine as the neuroleptic and appeared to do well. The neuroleptic was then changed to haloperidol. Five male and two female chronic schizophrenics received a combination of haloperidol, L-5HTP and MK-486 in a double-blind, crossover study.

Patients were stabilized for at least one month on an optimal dose of

haloperidol and an antiparkinson agent when necessary. After a month on haloperidol and placebo L-5HTP, patients began L-5HTP, 100 mg/24 hrs, along with MK-486, 200 mg/24 hrs. The intent was to follow a program of stepwise increments of L-5HTP dosage to achieve levels of 3 or more grams/24 hrs as in the previous study, but this turned out to be impossible. Of the 7 patients, 3 became so activated on 100 mg/24 hrs of L-5HTP that they had to be removed fr the protocol. All 7 patients showed some activation of illness in terms of their underlying pathology or character traits, but in 4 of the 7, these particular reactions subsided quickly, and permitted increased dosage of L-5HTP.

Preliminary data suggest that with each increment of L-5HTP, there was an increment of agitation or an exaggeration of existing pathology. There then followed in some patients an improved clinical status with better integration of thinking and more social interaction. This kind of improvement was vividly reported by 3 patients' families, who were, of course, unaware of the timing of the medication change.

These measurable clinical changes are striking, considering that such low doses of L-5HTP were used. We found earlier that without haloperidol, activation of symptoms occurred only when doses of L-5HTP of 2 to 3 grams per 24 hrs were reached. Tolerance to some side effects of L-5HTP developed in the patients during both studies. Transient parkinsonian side effects occurred in this study, whereas they did not at the much higher levels of 5HTP given in absence of haloperidol. It is clear that haloperidol in some way sensitized the central nervous system to exogenous L-5HTP.

Nine new chronic schizophrenic patients, three females and six males, participated in a second study of the clinical effectiveness of the drug combination 5HTP, chlorpromazine and MK-486. Doses up to 4 gm daily of 5HTP were given along with variable amounts of chlorpromazine. A common daily dose of chlorpromazine was 800 mg; however, patients received 200 mg MK-486/24 hrs. Although daily ratings of patients' attitudes and behavior are not yet available for analysis, no sustained clinical improvement was obvious in any of the patients. A preliminary impression is that 5HTP combined with chlorpromazine, like 5HTP and haloperidol, does elicit an increase in expression of affect. One patient in particular said repeatedly during active drug periods that she felt better, but no lasting change in paranoid ideas was seen. Apparently large doses of 5HTP in combination does not add much to therapy and may produce undesireable displays of irritability or uncontrolled affect in some chronic schizophrenic patients. An occasional patient may experience pleasurable affect on 5HTP without other marked change in clinical state.

B. Sleep Studies: Previously we found that chronic schizophrenic pati had a decreased number of rapid eye movements during REM sleep (REM density) and an increased number in NREM sleep. This situation is similar to one seen after the administration of parachlorophenylalanine (PCPA). PCPA decreases serotonin by inhibiting tryptophan hydroxylase. 5HTP reverses the sleep effect of PCPA-treated patients.

The sleep of 18 male, chronically hospitalized, schizophrenic patients was studied during prolonged 5HTP administration. This was compared to sleep studies of various lengths during the month-long placebo periods and immediately preceding and following 5HTP ingestion. During active drug administration, the peripheral decarboxylase inhibitor, MK-486, was also given. Seven of the 18 patients were studied at intermediate doses, 11 at high doses, and 7 during withdrawal from 5HTP.

EFFECT OF 5HTP ADMINISTRATION AND WITHDRAWAL ON MEAN SLEEP PARAMETERS

No. Subjects	P_1	5HTP (3600-5500mg) 7	P_1	5HTP (5600-6000mg) 11	P_1	Withdrawal 7
Total Sleep	318	274	335	273***	297	213
Stage II	223	209	219	18*	190	195**
Delta	6	0.04**	19	6***	28	11**
REM Latency	71	192	70	175***	91	19*
REM Time	78	52	86	69***	71	77
REM Density (Index)	1.4	1.6	1.5	1.6	1.5	1.7
REM %	23.2	19.4	24.3	25.2	21.9	36.2*

P_1- Sleep during the month placebo immediately preceding drug
Withdrawal - Sleep during the first week following last drug administration
*p<.05 (Wilson matched-paired signed rank test)
**p<.02
***p<.01
Values are in minutes/night except for REM % and REM Density

At low dosages of 5HTP (<2000mg) there was a significant increase in REM Latency (190 vs. 65), but no other changes in sleep. Total REM Sleep and Delta were significantly reduced on high dosages. Patients were agitated and sleep was poor during the first week of withdrawal; however, there was a significant increase in REM % and a decrease in REM Latency. During the second week of 5HTP withdrawal, all sleep measurements returned to baseline.

In addition, at the high dosages, the number of REM periods decreased compared to placebo (2.1 vs. 3.7, p<.01). Although the length of the first REM period was increased on high 5HTP dosages (43 vs. 18 min, p<.02), the time from onset of the first REM period to the second was unaltered.

The interpretation of the effects of 5HTP on sleep are complicated by such factors as dose, tolerance, and patient's behavioral response. Contrary to the hypothesis relating serotonin to sleep maintenance, total sleep decreased on 5HTP and contrary to the phasic-event suppression hypothesis, the REM density was not affected; however, REM density may not be the appropriate measurement of PGO spike density.

C. Biochemical: Serotonin is unique among neurotransmitters in that its receptors in vivo may be a ganglioside. Since gangliosides also specifically bind Vibrio cholerae enterotoxin, we are currently studying the effects of intracerebrally injected cholera toxin on 5HT metabolism in several brain areas.

Cholera toxin (1 μg/1 μl), injected stereotaxically into a 5HT cell body region, the ascending raphe nuclei, markedly elevates 5HIAA concentrations within 24 hours in the raphe nuclei, caudate nucleus, septal nuclei and hippocampus. The latter three areas contain 5HT terminals. Although the 5HIAA increase was between 60 and 100 percent, 5HT levels remained unchanged. Toxin injected in the lateral ventricle failed to affect 5HIAA levels in these brain areas.

Cholera toxin also markedly increased the accumulation of 5HT after pargyline when given 24 hours prior to the experiment. At the same time, the decline in 5HIAA was unaltered. These data suggest a stimulation of 5HT turnover by cholera toxin. The nature of this stimulation is currently under investigation in our laboratory.

III. The Catecholamines

AMPT (Alpha-Methyl-Para-Tyrosine)

Several lines of evidence suggest that certain types of schizophrenia may be associated with excessive dopamine activity. Psychotic symptoms in schizophrenics may be intensified by methylphenidate, which increases functional dopamine activity, and by L-DOPA, which is converted to dopamine. Amphetamine-induced psychosis in volunteers and in "speed freaks" is thought to be mediated by dopamine. The clinical efficacies of neuroleptics probably depend upon blockade of dopamine receptors. Alpha-methyl-para-tyrosine (AMPT) inhibits tyrosine hydroxylase, the rate limiting enzymatic step in dopamine synthesis. Although it should theoretically be clinically helpful in schizophrenics, two previous studies reported no effect when given alone. In a third study, however, Carlsson and his associates found that during treatment with AMPT, less thioridazine was required to treat schizophrenics. In this study, we report results of administering AMPT in a double-blind fashion to chronic schizophrenics who were concurrently treated with high doses of phenothiazines.

The subjects were 10 male chronic schizophrenics (age 19-35) who had been hospitalized for 2-15 years. Each received a constant dose of phenothiazines (average equivalent dose of chlorpromazine=800 mg/day). A four-week period of placebo administration preceded and followed by a three-week trial of AMPT (gm/day). Clinical effects were monitored by (a) the NIMH Nursing Rating Scale; (b) the BPRS administered by two psychiatrists weekly; and (c) the Comprehension Subscale of the WAIS before, during, and after AMPT administratic

As shown in the table, significant improvement was observed on the Comprehension Subscale ($p<.001$ compared with placebo periods) on AMPT. However, no

improvement was noted in the schizophrenia items of the BPRS or the NIMH Nursing Rating Scale, except for one patient. Clinical impressions of three, nonblind psychiatrists suggested moderate but transient improvement during the first week of AMPT administration in eight of 10 patients. Two patients developed symptoms on AMPT and one was withdrawn from the study. AMPT's effect on plasma levels of chlorpromazine (measured twice weekly) are presented in the table.

MEAN PLASMA CPZ (ng/nl)		
Placebo	77.7	
AMPT	72.9	p<0.01
Placebo	109.0	

AMPT markedly increased prolactin secretion (see table).

MEAN PLASMA PROLACTIN (ng/ml)		
Placebo	46.1	p<0.002
AMPT	111.6	p<0.01
Placebo	42.4	

MEAN (±SEM) SCORES ON FIVE CLINICAL OR PSYCHOLOGICAL VARIATIONS WITH AMPT

	Placebo	AMPT	Placebo	Analysis of Variance
Mean Psychosis Score (NIMH Inpatient Behavioral Rating Scale)	11.4±1.1	11.9±0.9	1.2±1.4	X^2_r = 4.20, NS
Mean Schizophrenia Score (BPRS)	9.5±1.2	7.5±1.1	8.4±1.1	X^2_r = 5.85, NS
Mean Comprehension Subtest Score (WIAS)	6.6±0.9	10.3±0.8	8.0±0.8	X^2_r = 11.56 (p<0.001)
Mean Depression Score (NIMH Inpatient Behavioral Rating Scale)	7.0±0.5	8.0±0.4	7.1±0.5	X^2_r = 5.45, NS
Mean Depression Score (BPRS)	5.9±1.3	4.8±1.2	4.8±1.3	X^2_r = 0.95, NS

The results suggest that during a three-week trial with AMPT, an inhibitor of dopamine synthesis, subtle improvement in psychological functioning (judgment and comprehension) appeared to occur in chronic schizophrenic patients on phenothiazines. Significant clinical improvement was not detected on the BPRS and NIMH Nursing Rating Scale. Since decrements on the Comprehension Subscal were produced in Parkinsonian patients by L-DOPA (Donnelly and Chase) these findings imply that excess dopamine activity may impair judgment and comprehension. Further studies will be required to clarify the clinical effect of AMPT in schizophrenic patients. These results will be important implications for the dopamine hypothesis of schizophrenia.

IV. Physostigmine

Ten patients have received oral physostigmine in varying degree in a controlled double-blind protocol attempting to replicate the earlier findings of Rosenthal that brief but dramatic clinical improvement could be observed during such treatment. None of the patients studied showed significant improvement. A report of this finding is in preparation. No further studies are contemplated.

V. Pineal Extract

Dr. Altschule reported in 1957 that injections of an aqueous extract of bovine pineal substance exerted a beneficial effect in chronic schizophrenia. A subsequent blind study on eight elderly, extremely chronic lobotomized schizophrenic women failed to show major therapeutic potential of the extract. We are testing the extract on a less chronically-ill population to see if there might be any merit to Altschule's earlier observations.

When the patients are clinically stable they are started blind on either a course of placebo or pineal extract. After 4-6 weeks on this medication, there is a crossover to the type material not already received. If the findings warrant, there is another shift of medication.

From the previous study done here using younger patients, it appears that there is a statistically significant but generally slight beneficial effect exerted by the extract on some patients. Only one patient can be said to have achieved a major remission associated with extract treatment. This finding has been confirmed in numerous crossover experiments.

A further three patients have been tried on pineal extract. Of the three one responded with significant clinical improvement. Six months after completing the protocol, this patient was given a second trial of pineal extract vs. placebo injections and again showed significant clinical improvement coincident with extract administration.

The most pronounced responder had been tried on enteric coated pineal tablets. Although an open study, the results were sufficiently encouraging to justify having more of these tablets made for further clinical trials, again in

selected patients. The advantage of administering the pineal substance in this form is that dosage can be increased. The earlier studies with intramuscular preparations was handicapped by the inherent volume limitations imposed by that route of administration.

Two additional patients have been tried on enteric coated pineal powder tablets. One became markedly aroused and hostile, with the other there was no change. The one patient already shown experimentally to be a marked responder to the aqueous extract and thought in an open study to be responsive to the oral preparation went through a double-blind placebo substitution. The patient showed worsening of her psychosis (as judged by the nursing ratings) while on placebo and improvement when active medication was substituted. Further extensive clinical testing of pineal materials does not seem justified at this time. Efforts are being made using experimental animals to develop techniques for either predicting who the few pineal responsive patients might be or what might be a possible mechanism of action. Until a working hypothesis along these lines can be developed, it seems more practical to test clinically only those few patients who have a clinical profile quite similar to that of the most responsive patient.

We were unable to isolate a fraction of the pineal extract that would be significantly enriched with regard to inhibitory activity on the in vitro assay of indoleethylamine-N-methyl transferase. Further study with pineal and liver extracts indicated that the inhibition obtained from extracts of these tissues was quite non-specific.

VI. Lithium Carbonate with Other Neuroleptic Drugs

There is good documentation in the literature indicating that chlorpromazine is superior to lithium in acute schizophrenic illness. The superiority of chlorpromazine in controlling extreme mania also appears to be established. Publications bearing on the use of lithium in schizo-affective illness are less unanimous in their conclusions. All of the studies so far reported do not indicate a superiority for the use of lithium over chlorpromazine in patients diagnosed as schizo-affective. There are no studies of the merits of using both medications simultaneously. It is our personal experience that many clinicians are using lithium carbonate in combination with major tranquilizers in the treatment of schizophrenic and schizo-affective patients, despite the fact that there are no controlled experiments in the literature bearing on the efficacy of this combination.

This study has been expanded and is taking place mainly outside the William A. White Research wards. This has been done for a number of reasons, among them our interest in being able to study a greater number of patients on low risk protocols such as this one. After several month's work, three wards in other services have been trained in the use of the rating instruments. In addition, one of the wards is in John Howard Division. This setting offers an excellent opportunity to study the effects of lithium ion on various aspects of aggression. The protocol has been appropriately modified for

this. Four patients have completed the protocol; five more are pending. Preliminary analysis of the data indicated that the clinical indications for combined treatment with lithium and phenothiazines are likely to be complex.

VII. Baclofen (Lioresal)

Last spring a brief clinical report appeared in Lancet stating that 13 treatment resistant chronic schizophrenics were found to improve and require less conventional neuroleptic medication when placed on Baclofen. Since Baclofen, then thought to be a GABA agonist but now thought to be an antagonist of substance P, is a compound in no way similar to currently used neuroleptic agents we have conducted a clinical trial. The results from the nine patients have been uniformly negative (see table). Despite the negative, this study will have important theoretical implications.

SYMPTOM CLUSTER

Psychosis		Arousal		Depression	
Placebo	Baclofen	Placebo	Baclofen	Placebo	Baclofen
14.3	14	11.2	11.7	6.8	7.2

VIII. Thyrotropin Releasing Hormone In Chronic Schizophrenia

Intravenous administration of synthetic thyrotropin releasing hormone (TRH) has been reported to lead to a prompt amelioration of schizophrenic symptoms in a pilot study involving four female patients. Drayson gave TRH to three patients with cyclical psychoses and failed to observe any clinical change. We gave TRH to three treatment resistant chronic schizophrenic males in an inpatient research setting in which all subjects were drug free for at least a month prior to study. Synthetic TRH, 600 μg (Abbott 38579) or matching placebo, was administered by slow intravenous injection at 8 a.m. for five days followed by a two-day holiday and then crossover to the treatment not given. Order of treatment was random; neither the person giving the injection nor any of the clinical staff was aware of the nature of the substance being given. The patient's clinical status was estimated by the nurses' rating scale as well as by clinical observation by the resident psychiatrist. Particular attention was paid to each patient the first day of each series of injections because of the reported rapid onset of clinical change. Statistical comparison of active TRH vs. placebo was by t-test of the means for appropriate questions for the five days of each treatment period. The results are summarized in the Table.

EFFECTS OF SYNTHETIC TRH ADMINISTRATION ON THE SYMPTOMS OF SCHIZOPHRENIA IN THREE CHRONIC SCHIZOPHRENIC PATIENTS. #: t-TEST

Patient # Treatment	Psychosis	Depression	Anxiety	Hallucinations & Delusions
Placebo	3.20±.09	1.53±.16	2.80±.15	1.35±.62
1) TRH	2.84±.25	3.18±.25	3.18±.22	2.20±.50
p	NS	.05	NS	NS
Placebo	3.47±.15	1.55±.30	2.68±.16	4.55±.28
2) TRH	3.23±.13	1.23±.20	3.10±.21	4.23±.38
P	NS	NS	NS	NS
Placebo	3.34±.12	2.28±.12	2.93±.24	3.80±.19
3) TRH	3.56±.14	3.53±.30	3.75±.12	5.43±.37
P	NS	.005	.01	.005

The table shows that two of the three patients showed some worsening of their clinical condition, particularly patient 3. It is especially interesting to note that the depression score for these two patients were significantly greater during the week on TRH as compared to the week on placebo. This finding stands in contrast to earlier reports suggesting that TRH has anti-depressant activity, and certainly TRH does not appear to be a useful drug for schizophrenia.

IX. Formation of Tryptolines (Tetrahydro-β-Carbolines) by Human Brain and Platelets

We have examined the products formed from tryptamine, N-methyltryptamine, and 5-hydroxytryptamine by an enzymatic preparation from either human brain or platelets, using 5-MTHF as a cofactor. In each case, we have demonstrated by TLC, co-crystallation, and gas chromatography/mass spectrometry that the reaction involves the addition of a carbon unit to the side-chain nitrogen, with subsequent cyclization to yield a tricyclic, tryptoline derivative. These compounds have been found in plants, but have not previously been associated with mammallian systems. For both enzymatic preparations, the optimal pH is 6.5 and phosphate buffers of increasing strength enhance the reaction. For the brain enzyme, methylcobalamine, flavin adenine dinucleotide, and ascorbic acid each enhance the reaction, while cupric ion inhibits it. We have also done preliminary investigations on platelet enzyme activities in normal volunteers. We found marked diurnal changes and wide variability among individuals; however, male enzymatic activities were significantly lower than those for females. These studies demonstrate that 5-MTHF does not act as a methyl donor, as previously believed.

We have examined the properties and distribution of the enzymatic reaction in the human brain, using 5-MTHF as cofactor. With indoleamines, activity increases with either hydroxy or methyl substitution at the 5-position of the

aromatic ring and decreases with the addition of methyl groups to the side-chain nitrogen. Both tryptoline and 5-hydroxytryptoline are substrates but 1-methyltryptoline is not. The enzyme has a similar Km for tryptamine and N-methyltryptamine and is inhibited by Cu^{++}, Ca^{++} and dithiothreitol. The enzymatic activity is unevenly distributed in the brain, with the highest activity in the hypothalamus and the lowest activity in the thalamus.

Acute ip injections of 5-hydroxytryptoline (5-HTLN) killed mice and rats between 12 and 48 hours post-injection with an LD_{50} dose of 320 to 350 mg/kg. Two weeks of daily injections of 200 mg/kg or 100 mg/kg 5-HTLN resulted in respective mortalities of 100% and 50%; surviving mice showed no changes in weight or gross appearance of brain, liver, lung, heart, spleen, kidney or adrenal. Neither acute nor chronic injections affected body weight or temperature except immediately before death where both dropped markedly.

In mice, one hour pretreatment with 200 mg/kg 5-HTLN ip, increased secobarbitol-induced "sleep time" (50 mg/kg ip). At these doses, neither drug, alone, is lethal; yet, 50% of animals treated with both drugs died 24-36 hours later. Intraventricularly, 10 µg of 5-HTLN to rats decreased motor activity for 25 minutes following the injection.

Acute application of 150-300 µg of 5-HTLN to the isolated rat fundus strip contracts the muscle in a manner similar to 2-4 ng of 5-HT. When added to the superfusion medium, 1.0 µg/ml 5-HTLN causes a tonic increase in muscle tone without blocking 5-HT-induced contractions. Methysergide blocks these 5-HTLN effects. Preliminary studies suggest that 5-HTLN may act on serotonergic mechanism in the brain.

A case of folate-responsive psychosis which was associated with a defect in $N^{5,10}$-methylenetetrahydrofolate reductase (methylene reductase) suggested the need to examine whether abnormally low activity of this enzyme might be of etiological significance in schizophrenia. We found that there was no significant difference in the platelet methylene reductase activity of chronic schizophrenics, compared to either hospitalized or nonhospitalized, age-matched controls subjects (see table). Although it is possible that a larger survey might reveal a subpopulation of schizophrenics who are characterized by abnormal methylene reductase activity, this study suggests that chronic schizophrenia is not generally associated with such changes.

Comparison of Platelet Methylene Reductase Activity in Schizophrenic Patients and Control Subjects

	N	Age (Mean ±SEM)	Methylene Reductase Activity (Mean ±SEM)*	Range*	r**
Normal Controls	15	28.7±0.94	0.86±0.12	0.15±1.36	-0.31
Hospitalized Controls	16	27.7±1.25	0.72±0 13	0.15±1.36	-0.32
Schizophrenics	18	28.1±1.19	1.17±0.18	0.05±2.28	-.35
All Subjects	49	28.1±0.65	0.93±0.09	0.01±2.28	-.31+

*Expressed as nmole/hr/mg protein; **Correlation of age to methylene reductase activity; +p<0.05

X. Sleep

The sleep of 13 drug free chronic male schizophrenics was studied during a one-month trial on chlorpromazine (CPZ) and compared with one-month placebo periods. Recordings were made only after subjects had been on the drug or placebo at least three weeks. Sleep Latency and awake time was significantly less on CPZ while Stage II, Delta Sleep, Delta %, Non-REM sleep, REM activity, and REM Density were significantly increased. There were no significant changes in REM Latency, REM time, REM %, Stage II %, and Non-REM %.

We investigated whether schizophrenics have a normal REM rebound following REM deprivation. In addition, since Cartwright et al. previously suggested that "good REM compensators" tend to be field independent, we related a measure of field independence (the portable rod and frame test) to degree of compensation.

Eight actively ill schizophrenic patients (E) (5F, 3M, mean age = 25) and eight non-psychotic control patients (C) (5F, 3M, mean age = 26) were partially REM deprived by the awakening method for two nights. Sleep on each of five Recovery nights (R_1-5) was compared with that of Baseline Sleep. All patients were unmedicated except for a single control patient who received chlorpromazine.

Mean Baseline Sleep patterns (min) were similar in E and C patient groups:

	Total Sleep	REM Sleep	REM Latency	REM %	Stage III + IV
E	378	93	72	25.0	37
C	390	99	78	25.5	32

Mean loss of REM was 71.8% for E and 72.8% for C. Controls had to be awakened significantly more frequently than E (35 awakenings compared with 28, p<.05).

On R_1 mean change in REM compared with Baseline was +38 min for C and -2 min for E (p<.01); on R_2 it was +17 for C and -10 for E (p<.05). Mean change in REM during all Five Recovery nights was +14.5 min for C and +1 for E (p<.05).

A two-way multivariate analysis of variance--one factor being patient groups, the other being a repeated measure, linear trend analysis--revealed that C had significantly different sleep patterns than did E during Recovery (p<.03, F=2.45). The major source of difference was in REM sleep recovery (p<.006, F=8.06) but, surprisingly, Stage III+IV was also a significant contributor (p<.02, F=6.13). E tended to have elevated Stage III+IV on the first

two Recovery nights and low and normal amounts on the last two except on the last Recovery night when Stage III+IV tended to be elevated, though not significantly.

REM rebounds during Recovery were correlated with increased Total Sleep ($r=.52$, $p<.01$). Nevertheless, after removing the effect of change in Total Sleep during Recovery by analysis of co-variance, the controls still had the normal pattern of REM rebound and the actively ill schizophrenic patients did not, i.e., the two groups differed in rate of REM decline over the five Recovery nights ($p<.03$).

Amoung four E and four C patients tested, there was a significant correlation between degree of REM rebound and field independence ($p<.05$, rho=.79). Mean change in REM during Recovery was 27 min for three field independent patients and -1.5 min among five field dependent subjects ($p=.036$, Mann-Whitney U-test).

These results suggest that actively ill schizophrenics differ from other psychiatric patients in failing to have a normal REM rebound. This difference was not attributable to differences in age, sex, baseline sleep, anxiety, or change in Total Sleep time during Recovery. REM compensation may also be correlated with field independence, though further research needs to be done to substantiate this finding before any conclusions may be drawn.

XI. Evoked Potentials and EEG

We studied the evoked potentials of 21 schizophrenics and 21 controls. They were matched for age and exposed to a 10-minute sequence of frequent and infrequent auditory stimuli. The amplitude of P_3, a response component of the evoked responses to infrequent stimuli, was much larger in the control group ($p<.0003$). Using a P_3 amplitude of 3.20 μV in the first 2.5 minutes as a dividing point, only 7 of the 42 subjects were misclassified. This amplitude had a significant correlation of 0.48 with the patient's age at first hospitalization. The group P_2 amplitude to frequent stimuli was also much larger in the control group ($p<.003$). This amplitude has a significant correlation of -.41 with medication dosage in terms of equivalent amounts of chlorpromazine. Behavioral scales failed to correlate significantly with evoked response measures. Evoked response latencies did not distinguish the two groups.

A study is underway to develop a means of demonstrating whether or not schizophrenic patients have an unusual lateralization of brain functioning. Visual processing of verbal and non-verbal tasks is being tested to show if patients have recognizalbe differences from normal subjects. The tasks invol the use of a projection tachiostoscope and are being presented to normal subjects to establish normal patterns of response. Patients are being tested also, but nonconsistent results have developed from limited trials. Should the theory behind this study prove workable, it could be applied to tests of various drug effects, or used to monitor the progress of treatment of patients in addition to providing information about a possible aspect of mental dysfunction.

XII. Brain Dopamine-β-Hydroxylase

Stein and Wise (1971) postulated that there might be a deficit in dopamine-β-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, in schizophrenic patients.

Wise, Baden and Stein (1974) tested their hypothesis by collecting the brains of 18 chronic schizophrenics dying in a state hospital and 12 persons without a history of psychiatric illness dying suddenly of heart attacks or accidents. The schizophrenics had decreased DBH activity ranging from 49 in the hippocampus, 58 in the diencephalon and 70 percent in pons-medulla. All these differences were statistically significant at $p<.05$. Because of these dramatic differences in the predicted direction, Wise et al. made a number of attempts to control for artifacts. After considering all these sources of artifacts, the large differences between schizophrenic and normal DBH activity remained.

We attempted to replicate their study. Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at Saint Elizabeths Hospital. Brain specimens from four chronic schizophrenics and nine controls were obtained by similar arrangement with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members, and hospital and police records. The control brains were obtained from periods without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The schizophrenics (7 males; 2 females) had a mean age of 49.2±6.3. Four died suddenly after traumatic suicides (in our experience, suicide is common among chronic schizophrenics), three from cardiac arrests, one from pulmonary aspiration, and one suddenly from pulmonary edema. The controls (all male) had a mean age of 42.3±4.5. Six died suddenly of trauma and three from cardiac arrests.

The periaqueductal pons-mesencephalon, hypothalamus and hippocampus were dissected out at autopsy and immediately placed on dry ice and subsequently stored at -80°C for up to one year. Wise et al. (1974) found DBH activity stable for one year at -15°C. There was no difference in mean storage time for the schizophrenics and controls. All DBH assays were performed by a staff member unaware of whether the samples were from schizophrenics or controls.

There was no significant difference in DBH activity in any area between the two groups. While the differences were not statistically different, DBH activity mean for the schizophrenic groups ranged between 77 and 89 percent of controls. Although seven of nine of our patients were taking phenothiazines at the time of death, Wise et al. (1974) indicate that this is probably not a cause of their DBH differences. Rats who had been given chlorpromazine (20 mg/kg 24 hour) for 12 weeks had a small increase in DBH activity. Although the patients in our study were taking a number of non-neuroleptic drugs, there was significant ($p<0.05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the

hypothalamus (r=-.60) and pons (r=-.65). This could mean that in the brains of schizophrenics, neuroleptics do decrease DBH activity, or possibly that there is a negative correlation between clinical pathology (as determined by the need for higher dosages) and DBH activity.

Wise et al. (1974) also attempted to determine the effects of post-mortem intervals on brain DBH activity (after a person dies, it may take several hours for that person to reach the morgue). Simulating this situation rats that were killed and allowed to remain three hours at room temperature prior to assay had DBH activity reduced by 27%. In a similar experiment performed in our Laboratory over a six-hour period, there was 15% decrease in activity. Since Wise et al. (1974) human brain post-mortem intervals were "several hours" and ours a mean of 4.2 for the controls and 3.0 hours for the schizophrenics, this does not seem to be an important factor, except possibly for one control who was not brought to the morgue for 27 hours. Since he had the lowest DBH activity of any of the subjects, the DBH activities were examined for the controls with and without this subject's values. The exclusion of this value did not change the failure to reach statistical significance. Two subjects with death-to-morgue times of seven and 11 hours had DBH activities about their group means. One subject with a 3.5 hour time lag was low. All other subjects had intervals of about one hour.

Wise et al. (1974) also compared the effects on brain DBH activity of the time from which the subject reached the morgue to the time of autopsy. They did this by storage of rat brains at 4°C for up to three days and found a decrease in DBH activity of 18%. Their subjects' brains, however, ranged from one day to eight days at this temperature. Because of this, they matched time intervals for controls and schizophrenics and still found the DBH activity of the schizophrenics lower. Our controls were in the morgue for a mean of 9.7±1.9 hours, while the patients were there for 35.6±7.9 hours, which is statistically significant ($t=2.73$, $p<.02$). Furthermore, there are significant negative correlations between the time in the morgue to autopsy and DBH activity in the hippocampus ($r=-.53$, $p<.05$) and hypothalamus ($r=-.49$, $p<.05$) while that for the pons ($r=-.46$) was not significant. This suggests that the uneven distribution between the two groups in morgue time may be responsible for the small non-significant differences in DBH activity in our samples. Since the Wise, Baden and Stein brains came from two sources, it is possible some of their group differences may be due to different storage techniques.

XIII. Dopamine Sensitive Adenylyl Cylcase

Striatal adenylyl cyclase activity from autopsied human brain is selectively stimulated by low concentrations of dopamine. Under the experimental conditions used, dopamine was about three times more potent than norepinephrin Histamine and serotonin were ineffective. This stimulation by dopamine was competitively inhibited by haloperidol. There was no difference between nine of the autopsied brains (head of caudate) of the control subjects without a psychiatric history and seven patients with chronic schizophrenia.

XIV. Biogenic Amines, Their Precursor Amino Acids and Their Metabolites

The role of biogenic amines in mental illness may include amines other than serotonin and catecholamines. In the present state of our understanding, little information is available on these amines. To bridge this gap, we have undertaken the task to develop comprehensive and sensitive mass fragmentographic methods to measure many of the known biogenic amines, their amino acid precursors and their metabolites.

Our intitial goal is to apply the method developed to measure the concentrations of the various amines and their derivatives in plasma, cerebrospinal fluid (CSF) and brain tissues. After establishing the normal ranges of these substances in biological materials, we hope to carry out several clinical and pharmacological studies in order to probe further the possible role of biogenic amines in mental illness.

The principal technique employed is mass fragmentography. To enhance the specificity of the method, selective gas chromatographic columns are used. These columns were chosen after extensive preliminary trials with various stationary phases and with different lengths of columns.

The biogenic amines, their amino acid precursors and their metabolites are partially separated and concentrated by either extraction into organic solvents or by thin layer or column chromatography. They are next dried down, derivatized to their appropriate pentafluoropropionate or trifluoroacetate derivatives and then injected into the GC column.

To correct for variations in processing the samples, internal deuterated isomers of the compounds of interest were snythesized, run through the whole procedure and then used to correct for any inconsistency.

The metabolites derived from tyramine, octopamine, dopamine and norepinephrine were extensively studied in the rat brain, lumbar and ventricular CSF. Mass fragmentography was carried out on the methyl ester/pentafluoroproprionate (ME/PFP) derivatives of the acidic and the PFP derivatives of the alcoholic metabolites, employing an 8 ft. 3% SE 52 column.

In nine patients with ventricular-atrial shunts (5 males, 4 females, age infancy to 65), ventricular metabolite concentrations (ng/ml, mean ± S.E.M.) were: p-hydroxyphenylacetic acid (22.4±3.6), p-hydroxymandelic acid (7.1±1), vanillylmandelic acid (3.1±0.3), dihydroxyphenylacetic acid (9.7±1.6), homovanillic acid (154±14.4), free 3-methoxy-4-hydroxyphenylglycol (MHPG) (11.7±1), conjugated MHPG (4.6±1.3), free p-hydroxyphenylethanol (PHPE) (12.3±1.7) conjugated PHPE (less than 2 ng/ml). The levels of the acidic metabolites in the lumbar CSF were all significantly lower than those in the ventricular CSF. The alcoholic metabolites on the other hand were not significantly different. P-hydroxyphenylglycol was also semi-quantitatively measured and found to be present almost completely in the free form.

Lumbar cerebrospinal fluid (CSF) of schizophrenic patients was studied.

Treatment with oral probenecid (100 mg/kg) increased the concentrations of p-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic and homovanillic acid by over three-folds compared to the baseline levels. The concentration of p-hydroxymandelic acid was also increased, but only moderately compared to the increase of homovanillic acid. Vanilmandelic acid showed a tendency towards being elevated by probenecid, but in our study, the elevation was not statistically significant, probably due to the large variance between subjects.

P-hdyroxyphenylacetic, p-hydroxymandelic, homovanillic, 3,4-dihydroxyphenylacetic, vanilmandelic acids and 3-methoxy-4-hydroxyphenylglycol were measured in control, acutely treated and ethanol-dependent rats.

Ethanol did not cause a shift from an oxidative to a reductive pathway in the metabolism of amines as is well established in the periphery. Ethanol was found to have no effect on the transport of the acidic metabolites across the blood brain barrier.

Metabolites derived from dopamine and norepinephrine were significantly elevated in both acutely and chronically treated rats while blood ethanol levels were high. During withdrawal (no ethanol was present in blood) only norepinephrine metabolites remained elevated in rats rendered dependent on ethanol.

The concentration of VMA and MHPG in whole rat brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8 percent. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 hr-1, respectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norepinephrine metabolism in brain.

The concentration of the free, glucuronide and the sulfate conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in human plasma, cerebrospinal fluid (CSF) and brain by mass fragmentography.

All three forms of MHPG were detected in the media analyzed. Free MHPG was found to be the predominant form in both the brain and the CSF. The sulfate conjugate of MHPG constitutes about 15% of the total MHPG in the CSF while in the brain the percentage varies between 30% in the hypothalamus and cortex and 80% in the substantia nigra.

The concentration of the glucuronide conjugate of MHPG measured in the brain and CSF represents about 5% of the total MHPG concentration.

In the plasma free MHPG and its glucronide and sulfate conjugates are present in about equal concentrations.

The relative concentrations of the three forms of MHPG measured in plasma, CSF and brain were compared with their concentrations in the urine from previously published results. From this comparison the diagnostic significance of each of the three forms of MHPG in the clinical assessment of central norepinephrine metabolism is discussed.

Human plasma concentratons of the major acidic metabolites of m- and p-tyramine, octopamine, dopamine, epinephrine and norepinephrine was measured. The free and conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were also measured. The means and ranges of concentrations of the acid metabolites were comparable except for m-hydroxyphenylacetic acid. Free, sulfate, and glucuronide conjugated MHPG were also found in about equal concentrations.

Vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in rat brain. The concentration of VMA and MPHG in whole brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8%. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 h^{-1}, repsectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norpinephrine metabolism in brain.

In a collaborative study with Dr. Norton Neff of the Laboratory of Preclinical Psychopharmacology, dopamine metabolism was studied in various regions of rat brain following the decline of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) from brain after treatment with pargyline, or from the accumulation of the acids after treatment with probenecid. The decline of DOPAC and HVA after pargyline treatment appeared exponential in all regions of brain studied with half-lives of about 13 min for HVA and 6.5 min for DOPAC. DOPAC was the major metabolite of dopamine, with various brain regions producing between 2 to 5 times more DOPAC than HVA. HVA accumulated after treatment with probenecid but the accumulation in one hour did not account for all of the HVA apparently eliminated from brain. DOPAC accumulated in some regions of brain (medulla, hypothalamus, and midbrain) and not in others (cerebellum, cortex, striatum, and hippocampus) after probenecid treatment. We conclude that dopamine metabolism is not uniform in brain and that the accumulation of DOPAC and HVA in brain after probenecid treatment only accounts for a minor action of the dopamine formed in brain.

Also in collaboration with the Laboratory of Preclinical Psychopharmacology a study was undertaken to measure phenylalanine hydroxylase activity in vivo

by measuring ^{18}O-tyrosine in plasma after exposing rats to ^{18}O. We were able to show that ^{18}O is incorporated in tyrosine in vivo and that the presence of ^{18}O-tyrosine in plasma is a relative measure of phenylalanine hydroxylase activity. Treatment with p-chlorophenylalanine decreases the ^{18}O incorporation into tyrosine. Because of the simplicity of the procedure and its easy adaptability to human studies, the incorporation of ^{18}O into endogenous constituents might serve as a useful diagnostic procedure for some metabolic disorders, for example, phenylketonuria. These results also indicate that labelling with ^{18}O cannot be used to measure the turnover rate of brain catecholamines as previously proposed.

We also studied dopamine metabolism and function in the rat superior cervical ganglion. Trans-synaptic modulation of dopamine metabolism in rat superior cervical ganglion was evaluated by contrasting normal and surgically decentralized ganglions. Dopamine metabolism was assessed from the decline of DOPAC after administering pargyline and from the concentration of dopamine and DOPAC after treatment with drugs. The concentrations of dopamine, HVA and DOPAC were about 19, 2.7 and 51 pmol/ganglion, respectively. DOPAC was the major metabolite of dopamine and its calculated rate of formation was 5.3 pmol/ganglion/min, which is approximately 300 nmol/g/hr. This is about 15 times faster than the rate of formation of dopamine reported for striatum. The rapid formation of dopmaine in ganglion was supported by the finding of a rapid decline of dopamine after ℓ-methyltyrosine treatment. In the same rat decentralization of the ganglion reduced the rate of formation of DOPAC, but increased DOPAC formation above normal in the intact sham decentralized ganglion suggesting that a feedback system may modulate dopamine metabolism. Treatment with carbachol alone or in combination with atropine of hexamethonium revealed that dopamine metabolism is modulated by nicotinic and muscarinic mechanisms. HVA and DOPAC accumulated in ganglion after treatment with probenecid indicating that an acid transport system is probably responsible for removing these materials from the ganglion. The similarities between the dopaminergic system of the ganglion and of the brain and the rapid rate of dopamine metabolism in the ganglion supports the notion that dopamine might serve as a transmitter or modulator of synaptic events with the ganglion.

XV. N,N-Dimethyltryptamine (DMT) and Phenylethylamine (PEA) In Rat Models of Schizophrenia

Two separate drug-induced behaviors in the rat that satisfy criteria which must be met for animal models of schizophrenia have been described. N,N-Dimethyltryptamine (DMT) and β-Phenylethylamine (PEA) were the two drugs studied because evidence suggests that they are viable candidates in the pathogenesis of schizophrenia. Both are endogenous compounds, found in human tissue. Both may be differentially elevated in psychiatric patients. When given to man, DMT produces psychological disturbance and amphetamine (almost structually identical to PEA) can produce a syndrome resembling paranoid psychosis. Instead of trying to find an animal equivalent of schizophrenia, we studied the behavioral effects of these compounds in rats and applied to these behaviors criteria derived from the clinical condition of schizophreni-

Both DMT and PEA produced dose response disruption of a well-trained conditioned avoidance response in the shuttlebox and PEA, with monoamine oxidase inhibition by pargyline, induced dose response stereotypy, associated with increased motor activity. The criteria we tested were alterations of the drug induced behaviors by acute or chronic neuroleptic pretreatment and tolerance development. Neuroleptics, which are efficacious in the treatment of schizophrenia, reduced DMT disruption and PEA stereotypy in a dose response relationship. In the case of DMT, disruption was attenuated to a degree directly related to length of neuroleptic pretreatment, consistent with long-term antipsychotic efficacy of neuroleptics. Pretreatment with dopamine antagonists and agonists suggests that the mechanism involved in DMT blockade may be through denervation supersensitivity of dopamine receptors. In the case of PEA, attenuation of stereotyped behavior paralleled the clinical potency of neuroleptics on a mg/kg basis and in terms of relative ratios. Complete tolerance did not develop to DMT disruption or PEA stereotypy, consistent with the chronic course of schizophrenia. However, rapid tolerance develops to PEA disruption of shuttlebox if doses subthreshold on PEA induced stereotypy. The drug induced behavioral models reported here are of particular interest because they involve endogenous compounds implicated in the pathogenesis of schizophrenia and there is some degree of pharmacological isomorphism between these models and the clinical condition of schizophrenia.

PEA in the Monkey

Six individually caged juvenile Macaca mulatta (Rhesus monkeys) were studied during two sessions before and four sessions after im injections of PEA. During each session, two observers, who were blind to treatment conditions, counted checking (a stereotyped form of hypervigilance manifested by abrupt changes in visual field) and 27 other discrete categories of behavior. Differences between pre-drug and post-drug behavioral counts were computed for each animal.

Single injections of PEA produced dose-related (25-75 mg/kg) significant increases in checking and lip smacking (another stereotyped behavior) as well as decreases in goal-directed behaviors such as grooming. These effects, which are often accompanied by variable increases in other stereotyped behavior such as rocking, chewing, gnashing and repetitive pacing were maximal at 15 minutes post injection, still substantial at 30 minutes, and mostly dissipated by one hour. These effects are similar to those induced by the chronic administration of amphetamines. Of interest is that unlike amphetamines, consistent tolerance did not invariably develop to the repetitive administration of PEA (75 mg/kg t.i.d.) for four or nine days.

Pretreatment with alpha-methyl-para-tyrosine (AMPT) (125 mg/kg), but not with phenobarbital (25 mg/kg) substantially reduced the characteristic effects of PEA. However, when the same AMPT pretreatment was followed by a subthreshold dose of L-DOPA (5 mg/kg) typical PEA effects were restored. This finding suggests that at least some PEA effects require intact dopaminergic mechanisms and that AMPT does not block PEA receptors.

The behavioral effects in Rhesus monkeys of endogenous substance PEA suggest that it may provide a useful animal model of psychosis.

XVI. Low Platelet Monoamine Oxidase Activity and Schizophrenia

The finding that blood platelet monoamine oxidase (MAO) activity is low in some schizophrenic patients has led us and others to inquire as to its significance. As part of this endeavor, there has been a continuous effort to determine if this low enzyme activity was due to one of the large number of artifacts that have plagued the investigation of biological factors related to psychiatric disorders. The enzyme has been assayed in the laboratory of Dr. Dennis Murphy, who, in addition to his interest in the enzyme in schizophrenia, has been using the platelet as a model for synaptosomes.

a. MAO Assay in Blood Platelets

In our initial studies, venous blood was collected in heparin, or more recently, in Becton-Dickinson Co. Vacutainer tubes containing ACD (acid-citrate-dextrose), NIH formula A, and platelets separated and stored according to previously described methods. MAO activty was determined using tryptamine-2-^{14}C HCl ($8x10^{-5}$M, 8.9mCi/mM) as substrate and was expressed as nM of tryptaamine converted per mg of platelet protein. The product was indoleacetaldehyde, whose formation was linear with time and enzyme concentration.

During the last several years our samples were also obtained in the ACD solution, but the MAO activity was determined in platelet rich plasma. The present assays uses benzylamine-^{14}C ($4x10^{-4}$M,4 mCi/mM) as substrate, and is expressed as nM of product per 10^{-8} platelets per hour. Benzylamine is a good substrate for both platelet MAO and an entirely different enzyme occurring in the plasma. Because of this, platelet rich plasma samples with and without the MAO inhibitor pargyline are run. Pargyline ($2.4x10^{-4}$M) completely inhibits the platelet enzyme without affecting the plasma enzyme. The value of sample with the inhibitor is subtracted from the sample without--the difference being the platelet activity.

The advantage of the platelet rich plasma assay over the previous one is in not having to determine platelet protein which can easily be contaminated with red and white cells.

b. Stability of Enzyme

Because at times it is not possible to process blood samples or run enzyme assays immediately, it is necessary to know the stability of the enzyme. Comparisons of blood samples processed immediately or kept on ice or at room temperature for five hours revealed no change in activity. Similarly, once the platelet rich plasma is separated, MAO activity remains stable for at least 30 days when kept at -70°C.

c. Comparison of Assays

Pearson correlations were run for 48 normals and 20 chronic schizophrenics between the two assays. The correlation for the normals was .85 while that for the chronic schizophrenic patients was .76. For the combined group the correlation was .83. All our platelet MAO activities are expressed in benzylamine values with a correction (derived from the normals) for values determined using the tryptamine assay.

d. Subjects

Normal bloods were drawn from normal volunteers and Hospital personnel at both the Clinical Center in Bethesda, Maryland and the William A. White Building of Saint Elizabeths Hospital. The bloods from the acute schizophrenics largely came from a research ward at the Clinical Center while those from the chronic schizophrenic patients came from special research wards at the William A. White Building.

e. Age and Sex

Others have reported that females had 10 percent higher platelet MAO activity than males. Our sample females have a slight but consistently higher MAO activity although this difference is statistically significant only for the young adult group.

f. Drugs

Although early experiments with chlorpromazine in intact systems indicated that chlorpromazine had some effects on amine degradation, this seemed to be due to poor amine accessibility to the enzyme because of alterations in the cell membrane. Edwards and Burns (1974) found that the I_{50} for amitriptyline was 2x10-4M for human platelet MAO in vitro when benzylamine was used as a substrate. The inhibition was competitive.

In studies on patients given neuroleptics, there does not appear to be a consistent difference between when they are on neuroleptics or off, or between patients on neuroleptics and their siblings not receiving drugs.

g. Hormones

One study using only two points found no alteration in platelet MAO activity during the human menstrual cycle. In another study which utilized samples collected three times a week, there were small (23 percent) peak to trough variations in activity in the majority of women. The peak MAO activity occurred during the preovulatory interval and the nadir occurred five to 11 days later. A small number of patients taking prednisone have been seen and have normal MAO activities. Similarly, there does not appear to be any correspondence between plasma thyroxin and triiodothyronine concentrations in psychiatric patients with low and normal MAO activities.

h. Other Factors

The hematocrit, hemoglobin and platelet counts, serum iron, and iron binding capacity were not systematically different in patients with low and normal MAO activity. Other platelet enzyme activities including succinate dehydrogenase, lactate dehydrogenase and cytochrome C reductase were no different in chronic schizophrenic patients with low MAO than they were in controls.

i. Chronic Schizophrenics

To date we have studied platelet MAO activity in 104 male chronic schizophrenic patients. Their mean ±S.E. MAO activity was 7.86±.56 while the value for 348 normal males (ages 18-50) was 11.04±.29. This difference, using a two-tailed "t" test, was significant at $p<.001$. The mean for the 15 female chronic schizophrenics was 7.04±1.06 while that for 332 normal females was 13.29±0.29 ($p<.001$).

The effect of chronic hospitalization on platelet MAO was studied at the Palo Alto VA Hospital where 50 normal controls were compared to 16 patients hospitalized for traumatic accidents to their spines. There was no difference between MAO activity between groups but 39 chronic schizophrenics had decreased platelet MAO activity.

j. Acute vs. Chronic Schizophrenics

To determine whether acute schizophrenics have a reduction in platelet MAO as did the chronic schizophrenics, platelet MAO of 27 acute schizophrenics were studied. The 10 male acute schizophrenics (ages 16-30) had a mean platelet MAO activity of 10.68±.78 which was no different than that of normal males. The 17 female acute patients had a mean of 13.85±1.12 which was no different from that for the normal females.

k. Subgroups of Chronic Schizophrenics

Platelet MAO in two groups of chronic schizophrenic patients (N=35, $\bar{x}$=7.52; N=42, $\bar{x}$=7.86) was significantly lower than in controls (N=680, $\bar{x}$=12.14) Values for those patients diagnosed as paranoid, (N=8, $\bar{x}$=4.47) were significantly lower when compared to non-paranoid schizophrenics (N=27, $\bar{x}$=8.43) in a retrospective study. These findings were confirmed in a separate prospective study of 42 chronic schizophrenics (paranoid and paranoid features N=24, $\bar{x}$=6.40 compared to non-paranoid N=18, $\bar{x}$=9.81). Schildkaut found low platelet monoamine oxidase to be associated with auditory hallucinations and delusion usually of a persecutory quality. Twelve out of 16 of these low monoamine oxidase patients were designated paranoid. Reduced platelet MAO activity in normal subjects appears to be associated with a greater tendency towards psychopathology and a perceptual behavior study which is similar to that of paranoid schizophrenics. These data could indicate that paranoid schizophrenia may represent a separate disorder from the other schizophrenias. The correlati

between paranoid schizophrenia and low platelet MAO activity suggests some biochemical difference between the different subgroups of schizophrenia.

l. Specificity with Regard to Other Illness

Since the various psychiatric diagnostic classifications are thought to represent different entities, a defect in one should not be seen in the other. The affective illnesses are the group we most diligently contrasted with the schizophrenias.

Sixteen male and 13 female unipolar depressed patients were found to have platelet MAO activity that was no different from our controls while 16 male and 33 female bipolar I depressed patients had reduced activity ($p<.001$). The bipolar patients had a negative skew which was not as large as that of the chronic schizophrenics. Using six units of MAO for the male normals and eight units for the normal females as a point of cut-off, a point under which less than 10 percent of normals fit, 39 percent of male chronic schizophrenics and 60 percent of the female chronic schizophrenics are found (chi square $p<.001$). The bipolar patients also differed from normals ($p<.001$ males; $p<.02$ females). The male bipolar patients were not significantly different from the male chronic schizophrenic patients, but the percentage of female chronic schizophrenics with MAO activity below eight units was greater than the female bipolar I patients. Thus, although bipolar I patients using the arbitrary cut-off have a mean MAO which is low, it is not clear whether they represent a separate group from the chronic schizophrenic patients.

m. Twins Discordant for Schizophrenia

To exclude the possibility that the low platelet MAO activity seen in the chronic schizophrenics was caused by nongenetic factors as opposed to genetic ones, we studied monozygotic twins discordant for schizophrenia. If the low platelet MAO activity is due to some aspect of being schizophrenic, low platelet MAO should only be present in the schizophrenic twin. Thirteen schizophrenic index twins, all of whom had been hospitalized at least once for schizophrenia and had been extensively studied by Dr. William Pollin and his associates, were examined along with their nonschizophrenic co-twins. At the time of the study, one patient resided within a hospital while five were in remission. Four of the patients had had acute forms of schizophrenia and six patients were not receiving any antipsychotic medication. The nonschizophrenic co-twins had never been hospitalized for a behavioral disorder and were functioning well within their families and communities, except for one individual with boderline psychosocial adjustment.

Only two twin pairs were living in the same household and nine of the co-twins were living in different cities. It was, therefore, necessary to obtain and prepare the blood samples at various facilities throughout the country. Because of this, normal control samples were obtained at the same time as those from the twins. They were coded and shipped to the Laboratory. All samples were batched together before assay. (In all studies samples are run

in a manner so that their origins are unknown to all investigators until the final calculations are made.)

The MAO activity of the 23 normal controls was the same as that obtained at that time for previous controls (16.02). The schizophrenic twins (9.88, $p<.005$) and nonschizophrenic twins (12.08, $p<.05$) were significantly lower than the normals, but there was no difference between the twin groups. There was a significant Pearson correlation ($r=.67$, $p<.01$) between the MAO activities in the schizophrenic and nonschizophrenic twins.

There were four pairs of twins whose MAO activities were especially low. The indexes of all four of these twin pairs were chronic schizophrenics while the more acute patients had MAO activities closer to normal. The severity of impairment (based upon number and duration of hospitalizations) was rated on a 5-point scale for the schizophrenic twins by an investigator with no knowledge of the platelet assay. A forced rank order was then made between the numerical ratings, with the highest number given to the most ill patients. Using this order, and comparing it to the MAO activities, a Spearman rank order correlation of -.54 ($p<.05$) was found.

Platelet MAO activity was also examined in nine monozygotic and 10 dizygotic normal twins. The intraclass correlation coefficient was 0.88 for the monozygotic and 0.45 for the dizygotic twins. The correlations were significantly different from one another (Mann Whitney U $p<.001$). Same-sex siblings matched for age and sex were about the same as the dizygotic twins while unrelated pairs were not significantly correlated with each other. These results are similar to those reported by Nies, Robinson, Lamborn and Lampert (1973) for normal monozygotic and dizygotic twins using benzylamine instead of tryptamine as a substrate.

Taken together this data indicates that platelet MAO activity is in a large part determined by genetic factors and that the low platelet activity seen in the chronic schizophrenics is not secondary to being ill, but is genetically related to the liability to be schizophrenic.

n. Family Studies

In order to further study the relationship of low platelet MAO to schizophrenia the blood of first degree nonschizophrenic relatives of index schizophrenics (N=16) was collected. Dividing schizophrenic indexes at their median, the low indexes had a mean of 4.64 and the high indexes had a mean of 10.81. The mean MAO activity for the low index-first degree relative was 8.56. The mean activity for high index-first degree relatives was 11.84. The difference between the means for the low vs. high first degree relatives was significant ($p<.02$; Mann Whitney U). No attempt was made in this small sample to account for sex differences. These differences between nonschizophrenic first degree relatives of high and low schizophrenics is another indication of the genetic nature of platelet MAO activity.

o. Physical-Chemical Studies

To date we have found no physical differences between the low and normal platelet MAO. The Km's from six schizophrenic patients using tryptamine as substrate (all below 8.37 units benzylamine) and from eight normals were nearly identical ($4.1x10^{-5}$ and $4.3x10^{-5}$M). The heat inactivation curves for both groups showed reductions in activity at 50°C which were not different for the two groups. The platelet MAO is not easily solubilized, which has, to date, prevented reliable electrophoretic studies. Dialysis of platelet sonicates from 10 schizophrenic patients and seven controls did not reveal any post-dialysis differences in activity between schizophrenic patients and controls.

p. Relationship of Platelet MAO in Brain MAO

The number of types of MAO's present in the brain is an unresolved question. Original studies indicated that there might be as many as four isoenzymes of MAO within the brain; however, Houslay and Tipton (1973) suggested that the multiple enzymes found using polyacrylamide-gel electrophoresis might be produced artifactually in a preparatory step. The authors state that these multiple MAO forms may represent a single protein with varying amounts of phospholipid attached; the amount of phospholipid may in turn determine the enzyme mobility during electrophoresis.

Another approach to this problem has come from the finding that selective inhibition of MAO can be used to identify multiple forms of MAO. Clorgyline was the first MAO inhibitor employed in this manner. It was found that tissue homogenates pretreated with increasing concentrations of clorgyline produced stepwise inhibition to the substrate tyramine, suggesting the presence of an enzyme sensitive to the inhibitor and one relatively resistent to it. Considerable more work has been done to identify these enzymes and for convenience they are called Type A and Type B.

The preferred substrates for the Type A MAO are norepinephrine, serotonin, and normetanephrine, while for the Type B they are benzylamine and β-phenylethylamine. Dopamine, tyramine and tryptamine are common substrates. Clorgyline, Lilly 51641, and harmaline are specific inhibitors of Type A while deprenyl and pargyline inhibit Type B. Most of the other MAO inhibitors used in clinical medicine, however, are nonspecific. Platelet MAO, forming one band on electrophoresis, appears to be a single enzyme with Type B characteristics. That is, the platelet enzyme demonstrates only a single sigmoid curve for inhibition using deprenyl, pargyline, or clorgyline. Furthermore, the platelet enzyme is 200-fold more sensitive to inhibition by deprenyl than clorgyline. The conclusion that the platelet enzyme is a simple Type B MAO is also supported by the high activity of the platelet enzyme with the relatively specific MAO-B substrates, benzylamine and β-phenylethylamine, in contrast to its lesser activity with serotonin, an A substrate.

q. Function

To determine whether or not the low platelet MAO activity might have a functional significance, platelet-rich plasma was obtained from 20 normal controls (age 29.6±1.1) and 16 chronic schizophrenic patients (27±1.4). Since plasma serotonin is almost entirely contained in the platelet the values were expressed in platelet units (ng/10^8 platelets). The platelet counts for the normals and schizophrenics were the same. When the patients had been off phenothiazines (N=12) for 30 days or longer the serotonin concentration was higher (80±4), while when the patients were taking phenothiazines (N=12) their platelet serotonin concentration was normal (90±9). The reason for the discrepancy between patients taking and not taking phenothiazines may be due to the latter's ability to block serotonin uptake into platelets. Nevertheless, since serotonin is not a good substrate for platelet MAO ($K_m > 10^{-3}$) other substrates might be much more affected.

r. Subclass of Schizophrenia

In 1974-1975 we undertook a retrospective study of 43 patients we had examined to look at who the patients were with low platelet MAO. Information was obtained regarding circumstances and age at onset of illness, presenting problems, diagnoses and family history of mental illness. The patient's medical and surgical history was reviewed, as well as laboratory data which included EEG's and skull x-rays. Mental status examination on admission and throughout hospitalization was summarized, as well as changes observed on medication. This information was gathered and coded without knowledge of platelet MAO activity.

Two types of statistical analyses were employed. The first approach correlated MAO activity with a variety of clinical variables using the Pearson product-moment correlation coefficient. The second approach looked for differences between groups of patients with low and high MAO activity. In the second approach, the MAO value of 9.16 units was arbitrarily chosen to divide the chronic schizophrenic patients into high and low MAO groups (12 patients in the high group; 31 patients in the low group). 9.16 is two standard errors above the female chronic schizophrenic mean (8.04) and slightly higher than two standard errors above the male schizophrenic mean (7.86). The term "high MAO" in this study refers to high MAO activity for chronic schizophrenic patients. The high MAO group has a mean ±SEM of 13.54±1.06, which is comparable to values for the normal population. The low MAO group had a mean of 5.47±.37. All patients were diagnosed chronic schizophrenics with a mean hospital stay of 9.3 years, although length of hospitalization within the sample did not correlate with MAO activity ($r=.11$). MAO activity did not correlate with age at onset of symptoms ($r=..06$), age at first hospitalization ($r=.12$), length of hospitalization ($r=.11$), or with problems at work ($r=.20$), school ($r=.07$), or marriage ($r=.12$).

Schizophrenic patients with low MAO had a history of more seizures ($r=.31$, $p<.05$) than patients with high MAO. Of the 41 patients from whom we had an

adequate history of seizures, 8 of 30 low MAO schizophrenics had at least one seizure during hospitalization, compared to 1 of 11 high MAO schizophrenics. The high-low difference was not significant using the Fisher Exact Probability Test. None of the patients was on anticonvulsive medication. The possibility of abnormal brain dysfunction correlating with low MAO was reflected in the significant negative correlation between MAO and the number of abnormal EEG's ($r=-.32$, $p<.05$). Of those 36 patients who had EEG records available, 10 of the 25 low MAO schizophrenics had at least one abnormal EEG, compared to none of the 11 high MAO schizophrenics (Fisher Exact Probability Test, $p<.01$). The data indicate that low MAO activity in chronic schizophrenics is associated with an increase in abnormal EEG's and perhaps an increase in seizures. This abnormal brain activity is, however, not associated with other medical or surgical problems, or with any abnormalities in routine blood studies.

In regard to the mental status examination, the patients with low MAO activity were reported to have more ambivalence ($r=-.32$, $p<.05$), but not any difference in serverity or frequency of thought disorder ($r=.02$), associational defects ($r=.10$), autistic behavior ($r=.03$), hallucinations ($r=.09$), instances of self-mutilation ($r=.04$) or suicide attempts ($r=.24$). No evidence for differences in treatment response related to MAO activity was found ($r=.05$). Mental illness in relatives did not correlate ($r=.19$) with platelet MAO activity and was not different between the high and low MAO groups. However, it might be impossible to determine such a correlation from the relatively small sample. This is compounded by the inherent difficulties in determining a complete accounting of family incidence of mental illness from chart analysis.

All of the paranoid schizophrenics had low MAO. None of the high MAO activity schizophrenics received a DMS II diagnosis of paranoid schizophrenia during their hospital stay; 7 of the 30 low MAO group did (Fisher Exact Probability Test $p<.05$.) This difference was further substantiated when the means of MAO activity were compared between those who received a diagnosis of chronic paranoid schizophrenia and those who did not. The paranoid schizophrenics have a mean MAO activity of $4.8\pm.46$, which is significantly lower than the nonparanoid schizophrenic values of $8.6\pm.75$ (t-test, $p<.03$). This difference was also reflected in symptoms characteristic of chronic paranoid schizophrenia, such as paranoid delusions and increased hostility, but not in other less specific symptoms.

From this study emerged a picture of a low platelet MAO activity schizophrenic who is more frequently diagnosed as paranoid, as having more paranoid type delusions, and as having abnormal brain activity reflected by increased incidence of seizures and abnormal EEG's.

In order to further examine these findings, 42 new patients were selected on the basis of definite diagnosis of schizophrenia who had active symptoms but without regard to subtype. Patients with a diagnosis of organic brain syndrome or mental retardation or other cerebral pathology, including severe alcoholism, were excluded in order to focus on the paranoid dimensions. The 42 subjects were drawn from a sample of 65. Fifteen were excluded because of

their history, eight others met criteria but refused to take part in the study. Seven of these eight refused because of apparent suspiciousness of distrust of the experimental procedure.

All patients met Research Diagnostic Criteria and 41 of 42 met Feighner's criteria for a diagnosis of schizophrenia. The mean age was 35, mean length of hospitalization was 401 months, and all patients had, at one time, received phenothiazine treatment. All patients had a chronic course and 40 of the 42 had a gradual or insidious onset of psychosis. Twenty-nine were males and 13 females. MAO values were 6.7 and 10.6 units for males and females, respectively, compared to mean MAO's of 11.04 and 13.29 for normal males and females. The difference between the schizophrenics and normals was significant at $p<.01$. The clinical staff who best knew the patient rendered a consensus diagnosis based on DSM II categories. A secondary diagnosis was reached for cases of chronic, undifferentiated schizophrenia. All patients underwent a semi-structured interview by research staff which included a shortened version of Wing's Present Exam and a paranoia checklist suggested by Rosenthal. Interviews were conducted at a time as close as possible to blood drawings, and for most patients were done on one occasions over a 60-mintue period. Repeated interviews were occasionally required to obtain the necessary information.

The information was gathered and coded without knowledge of MAO values. The data indicate that patients diagnosed as paranoid schizophrenics had a mean platelet activity MAO level of 5.4 for males and 8.9 for females, compared ith 8.6 and 12.1 for nonparanoid male and female schizophrenics. Patients with secondary paranoid features had MAO values of 5.6 for males and 9.1 for females. Values for both paranoid patients and patients with secondary paranoid features were significantly different from those of nonparanoid patients ($p<.01$).

The variables which best diagnostically divided the paranoid from nonparanoid patients by a linear discriminate function were: degree of suspiciousness, preoccupation with and systematization of delusions, delusions of control, stereotyped behavior, intact memory, rigidity of ideas, and communication characterized by evasiveness and misleading responses. These diagnostically discriminating items are similar to items on the Venables Scale for Paranoid Schizophrenics except for grandiosity which was not a good discriminator in our study.

These prospective findings confirm the retrospective chart analyses. Chronic schizophrenics had lower platelet MAO activity than normals. Paranoid schizophrenics had an even lower MAO activity level, whether the paranoia was determined by clinical diagnosis, by interview, or by a paranoia dimension scale.

s. Discussion

What does it mean to have low platelet MAO activity in view of seemingly

normal brain MAO activity? The argument that post-mortem measurement of brain MAO activity does not reflect its true activity because of autolysis has little support since brain MAO seems to be a very stable enzyme. It, of course, remains a possibility that we have either not measured brain MAO activity in the right region or with correct methods, an argument that is not easily dealt with.

Enzyme activity must now be measured in other regions of the body, such as the muscle, lung, liver, and kidney. If these areas turn out to have normal MAO activity while the platelet is low, intriguing explanations will be needed.

Part of the attractiveness of the concept of low MAO activity is that it is congruent with the two most generative biological hypotheses of schizophrenia. The first of these suggests that there is a functional excess of dopamine. A defect of MAO, an enzyme involved in dopamine metabolism, might be able to produce "dopamine excess." The second hypothesis suggests the formation of an hallucinogenic methylated indoleamine (transmethylation). Since the indolamines as well as their methylated congeners are metabolized by MAO, this theory is also consistent with a finding of low MAO activity. One of many questions that needs to be answered before low MAO activity can be considered causative, is "Why don't the vast majority of people given drugs which inhibit MAO become psychotic?"

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Barchas, J., Berger, P. A., Matthysse, S. and Wyatt, R. J.: The biochemistry of affective disorders and schizophrenia. In Clark, W. G. and del Guidice, J. (Eds.): Principles of Pharmacology (in press).

Stoff, D. M., Moja, E. A., Gillin, J. C. and Wyatt, R. J.: Insane rats in sane places: developing psychopharmcological models of schizophrenia. Proceedings of the Second International Rochester Conference on schizophrenia (in press).

Wyatt, R. J.: Is there an endogenous amphetamine: A testable hypothesis of schizophrenia. Proceedings of the Second International Rochester Conference on Schizophrenia (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01336-06 SMRC

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Longitudinal Studies of Schizophrenic Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas D. Reynolds — Chief, Section on Psychophysiology of Schizophrenia — SMRC

OTHER: NONE

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Psychopharmacology

SECTION

Section on Psychophysiology of Schizophrenia

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ongoing collection of daily behavior of 10 schizophrenics and three organic patients from WAW-4. Purpose is to explore evidence for homeostatic regulation in such data and to characterize such regulation mathematically and seek basis of differentiating (1) clinical groups and (2) changes occurring with treatment changes in individual patients. Controlling hypothesis is that behavioral steady states are more difficult for schizophrenics to achieve and our basis for his progressive withdrawal. Data suggests hebephrenics exhibit most marked behavioral cycles. Focus will be on comparing effects of varying psychotropic drug dosage on time-series parameters (phenothiazines and lithium).

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To obtain a quantitative description of behavior which permits empirical construction of predictive models. It is hoped that such models can be used as the units of an input-output theory of behavior, with particular reference to the problem of behavioral stability.

Methods Employed:

Behavioral notes are made 30 times daily on each of 14 chronic patients (12 schizophrenics, 2 organics for comparison purposes) on Ward 4 of the William A. White Building of Saint Elizabeths Hospital. The unit datum is a percentage of daily (or weekly) check-times positive for a given behavior (e.g., pacing, finger movement, talking, etc). The resulting time-series is examined in a variety of ways for evidence of non-random components. The models developed from the data are used to predict into new data, and progressively refined. When a model has been shown to be consistently superior to others, it is regarded as a better or more valid representation of the process being studied. Both discrete time series approaches and simulation techniques utilizing differential equations are being applied to the data.

Major Findings:

Work previously reported involved a variety of successful predictors for the time-series studied, but no uniform computational approach has been successful for all of the time-series. It become desirable, therefore, to develop an algorithm which would compete successfully with all previous individual predictors, so that each time-series could be meaningfully compared to another, for the purpose of stronger statements about comparisons across patient type.

At present, polynomial expressions with Gaussion weights have shown to be superior to all other types of expression. However, they are markedly superior to linear modeling only the cases where clear periodical signals are present. Non-stationarity has emerged as a critical problem in this property. Evidence to date suggests that non-stationarity more strongly characterizes the organic group (three patients) than the schizophrenic group.

Five to six-day behavioral cycles have been identified in three of the four hebephrenic patients on Ward 4 and in none of the non-hebephrenic patients. Power spectra are the most efficient techniques for detecting steady cycles. A publication is being prepared on this finding.

Preliminary data suggest that lithium may flatten the behavioral records of two patients with strong oscillations, corresponding to quantitative clinical improvement. This possibility will be systematically explored, since central to the entire study is the hypothesis of a homeostatic deficit in schizophrenia.

Simulation of one patient's cycle has been successful with a simple volterra scheme, and the method will be used on other data to explore drug response. Suggested hypothesis: phenothiazines increase efficiency of motor-discharge systems; do not reduce central excitatory state directly.

Significance to Bio-Medical Research and the Program of the Institute:

While the basic purpose of this study continues to be the ultimate development of input-output modeling of human behavior in specific environments, with particular reference to a study of disturbances of regulation in the major psychoses, it would appear that the overall methodology may be applicable to the study of response to psychotropic drugs, and perhaps yield some clues as to their mode of action in functional as opposed to biochemical terms. Overall strategy controlled by the hypothesis of a homeostatic deficit in schizophrenia.

Proposed Course:

The course for the next few years is fully determined by the above remarks. With the recent shift in data collection methods, we would expect to proceed in uniform fashion over an extended period of time, in order to seek reliable modeling data. Emphasis now is on the evaluation of drug response--a first "practical" test of the methodology.

Publications:

Reynolds, T. D.: Quantitative studies of schizophrenia. Behav.Proc. (in press).

Reynolds, T. D.: Mathematical analysis of a behavioral rhythm in a schizophrenic man. Waking and Sleeping (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01337-06 SMRC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Drugs of Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Wyatt	Chief, Lab. of Clin. Psychopharmacology	SMRC
	R. C. Stillman	Research Psychiatrist	SMRC
	J. C. Gillin	Research Psychiatrist	SMRC
	E. DeRenzo	Psychologist	SMRC
Other:	J. Eich	Psychologist	SMRC
	O. Wolkowitz	Psychologist	SMRC
	R. Willette	Chemist	NIDA
	R. Hawks	Chemist	NIDA
	P. Renault	Psychiatrist	NIDA
	N. Krasnegor	Psychologist	NIDA
	D. Murphy	Psychiatrist	LCS
	H. Weingartner	Psychiatrist	APB

COOPERATING UNITS (if any)
Division of Research, National Institute on Drug Abuse, Laboratory of Clinical Science, National Institute of Mental Health, Adult Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.85	2.5	1.35

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project continues to be determination of the mechanism of action of drugs of abuse and the reasons why these drugs are used. Early in our studies, we learned that chronic users of marijuana refused to take it when in groups in which they could carry on a good conversation. Their reason for this was unclear. More recently, we have found that information learned under marijuana is better remembered when again under marijuana then when straight and vice versa. Last year we showed that marijuana selectively interfers with the left hemisphere's ability to process information.

This year we showed that marijuana and alcohol differ in this respect. In a replication of previous findings, we found that marijuana depressed left hemisphere function while alcohol depressed both hemispheres.

Project Description:

Objectives:

To learn more about the mechanism of action and the reason why these drugs are abused.

Methods Employed:

Subjects were chronic marijuana users, usually adminstered natural marijuana cigarettes or a placebo cigarette prior to the biochemical or psychological testing.

Major Findings:

A. Interhemispheric Concomitants of Marijuana and Ethanol Intoxication

Marijuana may have a differential effect on the right and left cerebral hemispheres' ability to process certain kinds of information. Eight subjects after smoking marijuana assayed to contain 15 mg THC, showed an depression of left hemisphere reaction time superiority in the identification of tachistoscopically-presented pictures. We replicated these findings in two subsequent experiments and also found that alcohol depresses both hemispheres.

Numerous nonpharmacologic studies have shown that the right hemisphere is specialized in certain mental processes which are customarily believed to be enhanced by marijuana, such as the ability to perceive paralogical relations and connections, the appreciation of melodies, and the use of holistic (parallel-process) modes of thinking. Such studies have shown the left hemisphere in man to be specialized in activities frequently impaired by marijuana intoxication; e.g., logical, sequential thinking and verbal fluency. Our measurement of cerebral dominance, using tachistoscopic presentation of material to alternate visual hemifields, are the first to test the right hemisphere/left hemisphere effects of marijuana and alcohol directly. We plan to extend this study to include cocaine.

B. MAO Activity and Chronic Marijuana Use

We showed that platelet MAO activity is significantly lower in marijuana users than non-users, and lower in heavy users than light users. Acute marijuana smoking (single occasion) did not lower platelet MAO activity; therefore the lowered activity found in heaviest users is a chronic condition rather than an artifact of recent smoking. We plan to study this relation in a well-matched group of heavy users vs. a matched-control group of non-users.

C. Subjective Effects and Choice of Polydrug Use

We administered a structured interview and questionnaire to 100 consecutive subjects in our experiments to study their profiles of drug preference

and use. We have uncovered several highly significant relationships which may be quite important in understanding patterns of drug abuse.

We have found that the specific effects which a subject seeks from marijuana are very highly correlated with his choice of other drugs. Subjects who look to marijuana for increased insights, ideas, and a deeper involvement in music, people, etc., almost always prefer amphetamines to tranquilizers and barbiturates. Subjects who, on the other hand, use marijuana chiefly to relax strongly prefer the sedative/hypnotic drugs to stimulants. An interesting and important exception to this rule is cocaine, which is very much enjoyed, despite its stimulant qualities, by most of the sedative-seeking group. Another drug with considerable popularity in both groups is alcohol. We suspect that part of the appeal of marijuana, alcohol and cocaine is the ability of these drugs to create different effects depending on the user and the setting. Thus they are polyvalent drugs which can be used by different people in the same social setting to produce individually sought and varied effects.

D. Cooperative Studies of THC Analytic Methodologies

In collaboration with several NIDA contractors we have conducted cooperative validation studies which show RIA (radioimmunassay) techniques to be reasonably close in accuracy to much costlier GC/MS analyses of delta-9-THC in human blood.

E. Cocaine Mechanisms in Man

We will measure, using signal-detection techniques, the effects on vigilance and sustained attention of intranasally-administered cocaine. It is widely believed that cocaine sustains and augments one's ability to perform in tasks (physical and mental) in which fatigue would otherwise intervene. Signal detection methods will enable us to determine whether cocaine has any effect on (a) accuracy of detection of important information and (b) confidence in one's ability to perform, under conditions of rest and fatigue.

In addition, we will undertake a study of the acute effects of cocaine on memory and also look for state-dependent effects. Finally, we will see if cocaine actually "speeds up" two types of mental processes: visual scanning and internal scanning. Visual scanning will be tested using our corneal reflex recording equipment; one variable of interest will be the mean fixation time before and after administration of the drug. Internal scanning speeds, the amount of time needed to access material arranged in sequence on a list, will be tested using a Sternberg procedure. This will also provide an independent estimate of increased simple reaction time, should such occur.

Publications:

Stillman, R. C., Galanter, M., Fox, S., Lemberger, L., Weingartner, H. and Wyatt, R. J.: Metabolism and subjective effects of inhaled Δ^9-tetrahydro-

cannabinol under stress and no-stress conditions. Life Sci. 19: 569-576, 1976.

Eich, J. E., Stillman, R. C., Weingartner, H., and Wyatt, R.J.: Some effects of repetition on state-dependent accessibility. Proceedings of the American Psychological Association, 1976.

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Eich, J. E., Weingartner, H., Stillman, R. C. and Gillin, J. C.: State-dependent assessibility of retrieval cues in retention of a categorized list. J.Verb.Learn.Verb.Behav. 14: 408-417, 1976.

Wyatt, R. J., Cannon, E. H., Stoff, D. M. and Gillin, J. C.: Interactions of hallucinogens at the clinical levels. In Vesell, E. S. and Braude, M. C. (Eds.): Annals of the New York Academy of Sciences, 1976, pp. 456-486.

Cohen, S. and Stillman, R. C.: The Therapeutic Potential of Marijuana. New York, Plenum Press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01339-02 SMRC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Eye Tracking in Schizophrenic Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas D. Reynolds Chief, Section on Psychophysiology of Schizophrenia SMRC

OTHER: None

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION
Section on Psychophysiology of Schizophrenia

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eye-tracking of schizophrenic and normal controls is being explored. Pilot study suggests simple attentional deficits, correctable by making stimulus situation more interesting.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To attempt replication of reported finding of defective eye-tracking in schizophrenia.

Methods Employed:

Electrooculogram is recorded while subject follows a pendulum movement. Various stimuli are affixed to pendulum bob.

Major Findings:

Over 40 patients have been studied. Eye-tracking in schizophrenia most markedly improved by affixing picture of a face on the bob. Similar findings have been reported, but our data suggest schizophrenia tracking more closely approximate normal tracking than hitherto reported.

Significance to Bio-Medical Research and The Program of the Institute:

Utility of eye-tracking as a genetic marker rendered doubtful. However, as a sensitive technique for recording attentional deficit and the variables which influence the latter, the tracking task may be ideal. It will be pursued with more practical goals in mind (i.e., a technique for monitoring treatment response).

Proposed Course:

Present study has been completed and data being prepared for submission for publication.

We expect to continue to use apparatus as part of functional test battery used to monitor treatment response.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01183-04 SMRN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Interdisciplinary studies on neurons in vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Schlumpf	Ph.D.	SMRN NIMH
	W.J. Shoemaker	Ph.D.	SMRN NIMH
Other:	M. Sharp	Laboratory Technician	SMRN NIMH
	F.E. Bloom	M.D., The Salk Institute	
	A. Padjen	Ph.D., McGill University, Montreal, Canada	
	D. Forman	Ph.D., Bethesda Naval Med. Res. Inst.	
	D. Gruol	Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)

The Salk Institute, Davis Center of Neurobiology, LaJolla, California.

LAB/BRANCH

Laboratory of Neuropharmacology

SECTION

Comparative Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By micro-dissection of embryonic brain, norepinephrine-producing cells of the nucleus locus coeruleus and substantia nigra dopamine producing cells can be explanted and maintained in culture for several weeks. The growth of these brain stem explants are monitored by assaying for catecholamines (CA) with the sensitive radio-enzyme assay. More than half of the locus coeruleus cultures contain measurable (10 pg) quantities of norepinephrine and dopamine; reserpine added to the media (10^{-5}M) depleted both amines. Both brain stem cultures released large amounts of CA into the media. It was concluded that brain stem cultures synthesize CA at a very high rate. The cultured neurons develops normally by growing axons and dendrites, and by making synaptic contacts with each other, although the CA-containing neurons may have a deficit in storage capacity or regulation of CA synthesis. Nevertheless, locus coeruleus explant cultures are a suitable preparation for studies of the development and characterization of norepinephrine-mediate synaptic events.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01184-07 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)

Comparative neuropharmacology of the vertebrate cerebellum.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. R. Siggins	Ph.D., Acting Chief	SMRN NIMH
	B. H. Hoffer	M.D., Chief, Section on Developmental Neurobiology	SMRN NIMH
	S. J. Henriksen	Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)

D. J. Woodward, Univ. of Rochester, Rochester, N.Y.; S.C. Landis, Children's Hospital Med. Center, Boston, Massachusetts

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cerebellar Purkinje cells of many species show a similar chemosensitivity to putative neurotransmitters. Mutations which alter cerebellar connectivity in mice similarly do not change this chemosensitivity. This work suggests that postsynaptic receptivity of the Purkinje neuron develops intrinsically and early in phylogeny.

Project Description:

This project has been terminated.

Publications:

Siggins, G. R., Henriksen, S. J. and Landis, S. C.: Electrophysiology of Purkinje neurons in the weaver mouse: iontophoresis of neurotransmitters and cyclic nucleotides, and stimulation of the nucleus coeruleus. Brain Res., 114: 53-69, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01223-06 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Effects of stimulation of the locus coeruleus on Purkinje cells of the rat cerebelli.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.R. Siggins	Ph.D., Acting Chief,	SMRN NIMH
	B.J. Hoffer	M.D., Ph.D., Chief, Section on Developmental Neurobiol.	SMRN NIMH
	S.J. Henriksen	Ph.D.	SMRN NIMH
	F.E. Bloom	M.D., The Salk Institute	
	R. Freedman	M.D., University of Chicago	

COOPERATING UNITS (if any)
S. Landis, Children's Hospital Medical Center, Boston, Mass.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Office of the Chief, Laboratory of Neuropharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cells of the brain stem nucleus locus coeruleus (LC) are known to contain norepinephrine and project to several cortical regions. Activation of the pathway from LC to the cerebellar Purkinje cells by electrical stimulation results in inhibition of spontaneous firing, both in rat and mouse. The inhibition is accompanied by membrane hyperpolarization and a unique increase in membrane resistance. The inhibition is blocked by prostaglandins, by several phenothiazines, and by prior destruction of adrenergic nerves with 6-OHDA. The inhibitions are accompanied by an increase in cyclic AMP in Purkine cells, as detected by immunocytochemically. The responses to LC persist in the mutant mouse "weaver" , which lacks granule cells and parallel fibers, thus validating a direct monosynaptic action of the LC on Purkinje cells. This study was the first to show selective activation of central norepinephrine containing pathway, and thus provides a model for evaluation of psychoactive drugs on central monoamine systems.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01257-05 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The projections from the brain stem to the hippocampus of the rat.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. P. Oliver SMRN

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Office of the Chief, Laboratory of Neuropharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The experiments in this project are currently confined to the work in the hippocampal slice. In this preparation the synaptic transmission from known hippocampal inputs to pyramidal and dentate neurons are being studied in vitro to prepare a foundation for detailed studies of the effect of known transmitters on these classes of cells.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The slice preparation is used to characterize the interaction between known concentrations of various substances and the physiological properties of organized brain tissue.

Methods:

Hippocampi from guinea pigs are dissected out of the brain and sliced so as to maintain their anatomical structure. The slices are placed in a chamber designed for this purpose. The slice retains most of its normal physiology for several hours. The cells are spontaneously active and can be stimulated for experimental purposes. Quantities of drugs or other substances can be altered in the bathing medium.

Major Findings:

1. The slice can be made to generate epileptiform activity by penicillin application. 2. This activity can be used to screen the effects of anticonvulsant drugs in a quantitative manner. 3. The ionic mechanisms of epileptic activity can be studied in detail by this method.

Proposed Course:

One paper describing the effects of anticonvulsant drugs has been submitted to the Journal of "Epilepsia". A second paper on ionic mechanisms is in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01259-05 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Anatomy and function of norepinephrine pathways in the squirrel monkey brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S.L.Foote Ph.D.
A. Schwartz Ph.D.

Other: A.P. Oliver SMRN NIMH
F. E. Bloom M.D.

COOPERATING UNITS (if any)
The Salk Institute, Arthur Vining Davis Center for Behavioral Neurobiology.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Developmental Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of anatomical and physiological studies of the properties and functions of norepinephrine-containing neurons in the squirrel monkey brain. Specifically, we used histochemical neuronal pathway tracing techniques to demonstrate that there is a projection from locus coeruleus to many areas of neocortex in this primate brain. Further, we have utilized the technique of microiontophoresis in awake animals to determine that neurons in auditory neocortex decrease their spontaneous and stimulus evoiked discharge rates in response to local application of norepinephrine. We are currently using metal microelectrodes to record the discharge activity of locus coeruleus neurons during sensory stimulation and operant responding.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01295-04 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Technical methods for investigating the physiology and pharmacology of synaptic transmission.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. P. Oliver SMRN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Office of the Chief, Laboratory of Neuropharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The work in this project has been devoted to improving the microelectrodes used to study synaptic physiology and pharmacology at the single cell level. To date, instruments have been developed which permit measurements either intracellularly or extracellularly during the application of chemical substances intracellularly or extracellularly as a matter of routine.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objective of this study is to develop and improve technical methods and older ones used for the study of synaptic transmitters at the level of the single nerve cells. This is primarily accomplished by comparing the response of the cell to activation of a synaptic pathway with local application of the suspected transmitter substances by iontophoresis. Additionally, the pharmacology of the natural system may be tested with pharmacological agonists and antagonists at the single neuron level.

Methods Employed:

Several kinds of glass micropipette electrodes have been developed to study the activity of single nerve cells while either stimulating their natural synaptic input or applying pharmacological substances to them or to study the interactions of natural versus applied substances. More recently, techniques have been developed in which one electrode is glued to another in such fashion that one may be used to measure intracellular responses to applied substances. (Mr. Oliver received a Superior Performance Award for technical development achieved at this stage). To avoid the difficulties encountered with the gluing techniques a newer method has been developed and proven in which a diamond knife blade is used to cut holes at varying distances from the tip in some of the side barrels of pipettes with three or more barrels. One intact barrel is then used to record transmembrane potentials during extracellular drug applications from the others.

In addition to improvements in the micropipettes other equipment used to stabilize the tissue has been developed.

Major Findings:

The electrodes have been developed to the point where extra and intracellular measurement of synaptic activity and applied substances has become a routine laboratory procedure.

Significance to Bio-medical Research and the Program of the Institute:

Understanding the physiology and pharmacology of CNS depends on identification and characterization of transmitter action. The development of reliable technical methods is of considerable importance in obtaining this information.

Proposed Course:

The project is continuing to develop and improve the technical methods of single neuron studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01300-04 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
A Combined Electron Microscopic, Histochemical and Electrophysiological Study of Synaptic Innervation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: H. Weitsen Ph.D.

COOPERATING UNITS (if any)

Department of Anatomy, LSU Medical Center, New Orleans, Louisiana.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A combination of electron microscopic, histochemical and electrophysiological methods were utilized to investigate the synaptic organization of the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. The neurons were unipolar and were innervated by a single preganglionic axon. The cholinergic preganglionic fiber stained for acetylcholinesterase and was observed in both the light and electron microscope to spiral around the axon hillock of the ganglion cell before arborizing and making synaptic contact with the neuron. Most synapses were located on the soma near the axon hillock region, with electron microscopic features typical for cholinergic junctions. The axosomatic location of the synapses was manifested physiologically by a decrease in membrane resistance (increased conductance) at the peak of the fast EPSP and by a demonstrable reversal potential for the fast EPSP.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Synaptic transmission has been investigated in considerable detail in sympathetic ganglia. These ganglia are of particular interest because several types of synaptic potentials can be recorded, including a fast EPSP, a slow EPSP and a slow IPSP. The latter two are of particular interest because of their different mechanisms of generation. Much of our current understanding of the generation mechanisms of these synaptic potentials is derived from electrophysiological investigations on the ninth and tenth sympathetic ganglia of the bullfrog. There is, however, relatively little detailed morphological information on the synaptic organization of these ganglia. In view of this, we used a combination of histochemical and electron microscopic techniques to obtain additional information on the organization and fine structure of the innervation of these neurons. In addition, intracellular recording was used to study the physiological correlates of the synaptic organization.

Methods Employed:

In this study a combination of histochemical, electron microscopic and electrophysiological methods were used to study the ninth and tenth paravertebral sympathetic ganglia of the bullfrog.

For light microscopic histochemistry, the ganglia were excised, placed in a cryostat chuck and frozen quickly in isopentane cooled by dry ice. The tissues were maintained at a temperature of -22 C and sectioned at a thickness of 10-14 μM, placed on glass slides and air dried. The tissues were then fixed in 2-4% buffered formaldehyde and stained for acetylcholinesterase by the method of El-Bodawi and Schenk.

For electron microscopic investigation, the ganglia were fixed in situ by perfusion with buffered 3% gluteraldehyde and 1% formaldehyde. The tissues were post-fixed with 1% osmium tetroxide and subsequently prepared with standard procedures for electron microscopy. In experiments to determine the ultrastructural localization of AChE, an enzyme incubation step was added before treatment with osmium. Examination and photography of the thin sections were performed on Phillips 200, 300, and Zeiss 9S electron microscopes.

Intracellular recording in neurons of these sympathetic ganglia utilized standard electrophysiological techniques; the microelectrodes were filled with 3 M KCl. Preganglionic nerves were stimulated by means of suction electrodes on both the sym-

pathetic chain and the eighth spinal nerve. A similar electrode on the tenth nerve was used for antidromic stimulation. The ganglia were continuously superfused with oxygenated Ringer's solution.

Major Findings:

Light microscopic observation revealed the ganglion cells to be unipolar neurons. Acetylcholinesterase (AChE) activity was particularly localized to the region of the axon hillock and the adjacent neuronal soma. In some sections, the AChE localization was observed in a spiral around the axon hillock region of the neuron. The localization of AChE was also studied by electron microscopy. AChE was localized on the axolemma of the preganglionic axon and in the cleft around the synaptic terminals.

The find structure of the innervation showed that the preganglionic axon spirals around the axon hillock before making synaptic contact with the ganglion cell. The majority of synapses were found on the region of the soma near the axon hillock and on the axon hillock. The synaptic terminal had the electron microscopic appearance typical for cholinergic junctions.

The physiological correlates of the axosomatic location of synapses were studied by intracellular recording. In most neurons, the EPSP is all-or-none, indicating innervation by one preganglionic fiber. A test of membrane resistance shows that membrane resistance is markedly reduced at the peak of the EPSP. Additonally, electrical polarization of membrane reverses the EPSP at a membrane potential near -16 mV.

Significance to Bio-medical Research and the Program of the Institute:

The combination of anatomical and physiological techniques used in this investigation has provided information on the synaptic innervation of the sympathetic ganglion cells of the bullfrog. The all-or-none characteristics of the EPSP indicates that the neurons are innervated by one preganglionic fiber. The acetylcholinesterase histochemistry and the electron microscopy show that the preganglionic fiber spirals around the axon hillock, arborizing and making synaptic contacts on the soma of the neurons, particularly near the axon hillock. Since, in most neurons, the action potential originates in the region of the axon hillock, this anatomical location of synapses appears particularly well suited for the synaptic activation of action potentials.

The location of synapses on the soma and axon hillock regio

of these sympathetic neurons makes these neurons particularly well suited for investigating the ionic basis of synaptic potential generation. Frequently, the conductance changes underlying the generation of synaptic potentials cannot be adequately investigated in vertebrate neurons because the synaptic junctions are remote from the soma. As a result, it may be difficult or impossible to detect a conductance change associated with the EPSP. Furthermore, it also may not be possible to determine a clear cut equilibrium or reversal potential for the EPSP. As shown in this investigation, the neurons in sympathetic ganglia of bullfrogs are monopolar and do not have dentrites. Additionally, the synapses are predominantly located on the soma. One would therefore expect to be able to demonstrate the conductance changes generating the synaptic potentials in these neurons. The physiological investigations in this project confirm that this is indeed the case. The increase conductance that generates the fast EPSP is demonstrated by the decrease in membrane resistance at the peak of the fast EPSP. Furthermore, the somatic location of synapses is also manifested by the ability to reverse the fast EPSP by electrical polarization of the membrane. On the basis of the combined anatomical and physiological data in this study, it is concluded that the sympathetic ganglion of the bullfrog is an excellent preparation for investigating transmission at neuronal synapses.

Proposed Course:

The continuation of the project will be directed toward further elucidation of the synaptic organization of these ganglia in relation to the generation of different types of synaptic potentials.

Publications:

Weitsen, H.A. and Weight, F.F.: Synaptic innervation of sympathetic ganglion cells in the bullfrog. Brain Res. 128: 197-211, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01302-05 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Function of the dopamine projection to rat caudate nucleus; possible involvement of cyclic AMP.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. R. Siggins	Ph.D.,Acting Chief	SMRN NIMH
	B. J. Hoffer	M.D.,Ph.D., Chief, Section on Developmental Neurobiology	SMRN NIMH
	S. J. Henriksen	Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)

U. Ungerstedt, Karolinska Institute, Stockholm, Sweden

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Caudate neurons of rat were tested by iontophoresis. Dopamine, apomorphine, cyclic AMP, ATP, 5'AMP, adenosine and 2-Cl-adenosine all inhibit the spontaneous firing of caudate neurons. Results of studies using interactions of these agonist responses with phosphodiesterase inhibitors, phenothiazines methyl xanthines and prostaglandin E1 all suggest that these neurons possess two indepenent inhibitor receptors both of which may generate cyclic AMP postsynaptically. That a tonically firing inhibitory dopamine pathway projects to caudate from substantia nigra is suggested by studies with 6 OHDA, which removes this pathway.

Project Description:

This project was terminated with the resignation of the PI.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01303-04 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)

Studies on Physiological Mechanisms Modulating the Release of Synaptic Transmitter.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: S. D. Erulkar Ph.D.

COOPERATING UNITS (if any)
Department of Pharmacology, University of Pennsylvania Medical School, and Marine Biological Laboratory, Woods Hole, Massachusetts.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of repetitive action potentials in the postsynaptic axon on the release of synaptic transmitter from the presynaptic terminal was investigated at the squid giant synapse. Repetitive antidromic stimulation of the postsynaptic axon resulted in a reduction in EPSP amplitude. The reduction in transmitter release was accompanied by a decrease in the presynaptic spike after-hyperpolarization (AH). Increasing the concentration of extracellular potassium ions also reduced the EPSP and decreased the amplitude of the presynaptic spike AH. The reduction in transmitter release resulting from repetitive postsynaptic impulses is attributed to the accumulation of extracellular potassium ions. The accumulation of extracellular potassion ions may thus modulate synaptic transmission and function as an integrative mechanism in the nervous system.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The modulation of synaptic transmitter release may play an important role in the processing of information in the nervous system. Mechanisms which have been reported to modulate synaptic transmission include repetitive activity in the presynaptic terminals and the presynaptic action of other synapses. There has been, however, no evidence for a direct postsynaptic role in the modulation of transmitter release. This fact, together with evidence that increasing the extracellular potassium (K) concentration depressed transmitter release, prompted us to investigate whether the accumulation of extracellular K resulting from a train of postsynaptic impulses might modulate transmitter release.

Methods Employed:

The stellate ganglion was dissected from the squid, Loligo pealii, and placed in a small Lucite chamber perfused by oxygenated artificial seawater. The bath temperature was lowered until the EPSP no longer initiated spike generation. Experiments were then performed at a constant temperature ($\pm$ 0.1° C) between 6 and 12 C. Standard electrophysiological techniques were used to record from microelectrodes placed intracellularly in the midregion of the presynaptic terminal and in the postsynaptic axon near the synapse.

Major Findings:

Repetitive antidromic action potentials in the postsynaptic axon produced a depression in the release of synaptic transmitter from the presynaptic terminal. Following a train of postsynaptic action potentials, EPSP amplitude was reduced. The average percentage reduction in EPSP amplitude was 27.6 $\pm$ 7.9 (s.d.m.; n=9) for postsynaptic stimulation at a frequency of 25 Hz for 20 seconds.

The repetitive impulses in the postsynaptic axon also reduced the amplitude of the presynaptic spike AH. In five experiments, when the ratio of change in presynaptic spike AH to change in postsynaptic spike AH was compared for different stimulus durations, the ratio for a given synapse varied by less than 0.1. These data indicate that the K that accumulates in extracellular spaces around the postsynaptic axon also affects the presynaptic terminal.

Increasing extracellular K also produced a reduction in the amplitude of both the EPSP and the presynaptic spike AH. On the average, increasing extracellular K resulted in a 20% change in EPSP amplitude per mV change in presynaptic spike AH. With repetitive antidromic stimulation of the postsynaptic axon, the

average ratio of percentage change of EPSP amplitude to change of AH was 27.6 ± 4.3 (s.e.m.; n=31). This suggests that the reduction in EPSP amplitude resulting from repetitive postsynaptic action potentials is due to the accumulation of extracellular K ions around the presynaptic terminal.

Significance to Bio-medical Research and the Program of the Institute:

The modulation of transmitter release by repetitive postsynaptic action potentials can function as a type of negative feedback to inhibit impulse transmission through a synapse. Previous investigations have shown negative feedback through synaptic inhibition of an output neuron, but not a direct feedback on synaptic input. This investigation provides evidence for a direct inhibitory feedback from the postsynaptic structure that modulates transmitter release from the presynaptic terminal. In this way, the accumulation of extracellular K resulting from physiological activity may modulate synaptic transmission and function as an integrative mechanism in the nervous system.

Proposed Course:

The continuation of the project will be directed toward elucidating other physiological mechanisms involved in the modulation of synaptic transmitter release.

Publications:

Weight, F.F. and Erulkar, S.D. Modulation of Synaptic Transmitter Release by Repetitive Postsynaptic Action Potentials. Science 193: 1023-1025, 1976.

Weight, F.F., The Modulation of Synaptic Transmission and Information Processing in the Nervous System. In: IEEE, Conference on Cybernetics and Society, 1976, pp 285-299.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01304-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Role of Potassium in the Control of Synaptic Transmitter Release.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
Other:	S. D. Erulkar	Ph.D.	

COOPERATING UNITS (if any)
Department of Pharmacology, University of Pennsylvania Medical School, and Marine Biological Laboratory, Woods Hole, Mass.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of changes in extracellular potassium (K) concentration on synaptic transmission were studied at the squid giant synapse with intracellular recording from the presynaptic terminal and the postsynaptic axon. The amplitudes of both the presynaptic spike and the EPSP varied inversely with extracellular K. On the average, a 10 mV change in spike height was accompanied by a 3.1 mV change in EPSP amplitude. The amplitude of the presynaptic spike after-hyperpolarization varied inversely with extracellular K. On the average, increasing extracellular K resulted in a 20% change in EPSP amplitude per mV change in presynaptic spike after-hyperpolarization. The effect of extracellular K on EPSP amplitude appears to be mediated by changes in the amplitude of the presynaptic spike.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Potassium (K) ions have been shown in previous investigations to accumulate extracellularly with repetitive nervous activity. There is little information, however, on the functional significance of such increases in extracellular K. In this investigation, we studied the effect of changing extracellular K concentration on synaptic transmission.

Methods Employed:

The experiments were conducted on the giant synapse of the squid, Loligo pealii, at the Marine Biological Laboratory in Woods Hole. The stellate ganglion was dissected, placed in a small lucite chamber and constantly perfused with oxygenated sea water. Artificial sea water with a K concentration of 9 mM was changed by addition to or substraction from the artificial sea water, without adjustment of the other ions. Postsynaptic spike initiation was prevented by cooling the preparation sufficiently to reduce the EPSP below threshold.

The recording microelectrodes were filled with 3 M KCl. One microelectrode was placed in the postsynaptic giant axon close to the synapse, the other electrode in the mid-region of the presynaptic terminal. Standard electrophysiological techniques were used for recording. Traces on two oscilloscopes were photographed on separate cameras. Measurements were made from enlarged photographic images on an analog to digital converter (Oscar, Model F). Calculations were performed on a PDP-12 computer. Least squares analysis of exponential functions were performed on a PDP 10 computer.

Major Findings:

An increase in extracellular K from 9 mM in the artificial sea water (ASW) to steady levels of 10.5 mM and 12 mM, progressively decreased the amplitude of the EPSP. Conversely, decreasing extracellular K to 6 and 3 mM progressively increased the amplitude of the EPSP. Within the range of extracellular K tested (3-12 mM), the relationship between extracellular K and the percentage change in EPSP amplitude was linear; a 24.5% change in EPSP amplitude occuring per mM change in extracellular K.

Since K conductance is high during the spike after-hyperpolarization (AH), the membrane behaves as a K electrode and the amplitude of the AH can be taken as an index of extracellular K concentration. The ratio of percentage change in EPSP to change in AH can therefore be used to assess whether a change of transmitter release results from the

accumulation of extracellular K. This relationship for increasing extracellular K gives a 20% change in EPSP amplitude per mV change in presynaptic spike AH.

One mechanism that could account for the effect of extracellular K on EPSP amplitude is that it may be mediated by changes in the amplitude of the presynaptic spike. Such presynaptic changes were observed. There was a 3.1 mV change in EPSP amplitude per 10 mV change in spike height. The results of the present experiments indicate that the change in EPSP amplitude produced by changing extracellular K can be accounted for by the change in presynaptic spike height. The relationship between presynaptic spike height and EPSP amplitude agrees essentially with data from experiments by others on the same preparation using different techniques to alter the amplitude of the presynaptic spike.

Significance to Bio-medical Research and the Research of the Institute:

Although repetitive action potentials are known to result in the accumulation of extracellular K in nervous tissues, there is little information on the physiological effects and functional significance of increased extracellular potassium. This investigation shows that the effects of changes in extracellular K concentration on synaptic transmission. Increased extracellular K can depress synaptic transmitter release from presynaptic terminals. This could be important functionally when nervous activity results in an accumulation of extracellular K.

Proposed Course:

The continuation of the project will be directed toward an understanding of the effects of extracellular K on synaptic transmission at other junctions.

Publications:

Erulkar, S.D. and Weight, F.F., Extracellular potassium and Transmitter Release at the Giant Synapse of Squid. J. Physiol. 266: 209-218, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01305-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
The Role of Temperature in Synaptic Transmitter Release.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: S. D. Erulkar Ph.D.

COOPERATING UNITS (if any)
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Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington,D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lowering the temperature of the squid giant synapse prevents EPSP initiation of the postsynaptic action potential and permits investigation of the relationship between the EPSP and the presynaptic action potential. This study revealed that transmitter release is temperature sensitive; from an Arrhenuis plot, an apparent activation energy of 74 kcalorie per degree, was calculated. A change in presynaptic spike height does not appear to be the major factor involved in the sensitivity of transmitter release to temperature.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

There have been several previous investigations of the effects of temperature on synaptic transmitter release at different synaptic junctions. Although all reports agree that synaptic transmission is affected by temperature, detailed analysis of the factors involved has not been possible because the relationship between presynaptic events (such as spike amplitude) to postsynaptic response could not be investigated. It is possible to study this relationship at the squid giant synapse where intracellular recording can be obtained from both the presynaptic terminal and the postsynaptic axon. In this study we used to squid synapse investigated the effect of temperature on the presynaptic spike and the EPSP.

Methods Employed:

The stellate ganglion was dissected from the squid, Loligo pealii, placed in a small Lucite chamber and perfused continuously with oxygenated seawater. The temperature of the bath was controlled by a thermoelectric device and continuously monitored. Micropipette electrodes filled with 3 M KCl were inserted in the mid-portion of the presynaptic terminal and in the postsynaptic axon near the synapse. Conventional electrophysiological recording techniques were used.

Major Findings:

At room temperature (18-20^{o}C), the EPSP in the squid axon almost always generates a postsynaptic action potential. In all 43 synapses investigated, when the temperature was lowered sufficiently, initiation of postsynaptic spike generation by the EPSP did not occur. The mean temperature at which the EPSP failed to activate a spike was 8.9 $\pm$ 1.5 (s.d.m.) and the range was from 5.6 to 12^{o}C.

When temperature was decreased below the point at which the postsynaptic spike was activated, EPSP amplitude decreased progressively. This was accompanied by an increase in the width of the presynaptic spike due to a decrease in the rate of rise and fall of the action potential. Additionally, an increase in synaptic delay was also observed.

The relationship between temperature and the log of EPSP amplitude was linear with a high degree of temperature sensitivity. From an Arrhenius plot of the log of EPSP amplitude against the reciprocal of temperature (K), an apparent activation energy of 74 kcalorie per degree was calculated.

The greatest change in EPSP amplitude was in the temperature range between 9 and 10 C. In contrast to this, the largest change in presynaptic spike height was in the temperature range between 6 and 8 C. The comparison between change in presynaptic spike height and EPSP amplitude, as a function of temperature, does not agree with this relationship reported in previous studies using electrical polarization of the presynaptic terminal. This suggests that changes in the presynaptic spike height may not be a major factor in the effect of temperature on transmitter release.

Significance to Bio-medical Research and the Program of the Institute:

It is important to elucidate the mechanisms by which the release of synaptic transmitter from nerve terminals is controlled, in order to understand the cellular basis of nervous system function. In previous investigations on the effects of temperature on transmitter release, it has not been possible to study presynaptic mechanisms. Using the squid giant synapse in order to study presynaptic release mechanisms, we found that a change in presynaptic spike height does not appear to be the major factor involved in the sensitivity of transmitter release to temperature. The effect of temperature on transmitter release is therefore likely to involve other factors such as temperature sensitivity of the release mechanism.

Proposed Course:

The continuation of the project will be directed toward understanding the molecular mechanisms involved in transmitter release, as elucidated by these temperature sensitivity experiments.

Publications:

Weight, F.F. and Erulkar, S.D.: Synaptic transmission and effects of temperature at the squid giant synapse. Nature 261: 720-722, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01306-01 SMRN

PERIOD COVERED

July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)

Fluorescence Histochemistry and Electron Microscopy of Small Intensely Fluorescent (SIF) Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluorescence histochemical and electron microscopic techniques were used to investigate small intensely fluorescent (SIF) cells in the ninth and tenth paravertebral sympathetic ganglia of bullfrogs. Fluorescence histochemistry revealed that clusters of SIF cells are sparsely distributed in the ninth and tenth ganglia; the clusters were usually located in the vicinity of blood vessels. Fluorescent processes were not observed emanating from SIF cells. The clusters stained positively for the chromaffin reaction indicating that SIF cells are chromaffin cells. Ultrastructurely, the SIF-chromaffin cells in the sympathetic ganglia appeared virtually identical to the chromaffin cells in the adrenal gland; this includes two cell types that appear morphologically the same as the epinephrine and norepinephrine containing chromaffin cells in the adrenal gland. Efferent synapses from the SIF-chromaffin cells in sympathetic ganglia to sympathetic neurons were not observed. The SIF-chromaffin cells in bullfrog sympathetic ganglia did not have the morphological characteristics of interneurons; it is suggested that they may function as extra-adrenal chromaffin tissue.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Small intensely fluorescent (SIF) cells have been reported in the sympathetic ganglia of several species. On the basis of electrophysiological studies on rabbit superior cervical ganglia, it has been proposed that SIF cells may be adrenergic interneurons in the pathway that mediates the slow IPSP. The bullfrog sympathetic ganglion is particularly well suited for investigating the morphologic basis of the slow IPSP because it is generated in a separate cell type by stimulation of a specific synaptic pathway. Furthermore, much of our current understanding of the mechanism of generation of the slow IPSP derives from investigations on bullfrog sympathetic ganglia. There is, however, relatively little morphological information on the synaptic organization of this preparation. We therefore initiated this investigation in an attempt to demonstrate morphologically the specific anatomical pathway mediating the generation of the slow IPSP.

Methods Employed:

Small intensely fluorescent (SIF) cells were identified using the Falck-Hillarp fluorescence method for the cellular localization of biogenic amines. Sympathetic ganglia were excised and frozen in isopentane cooled with dry ice. The ganglia were freeze-dried for 3-5 days and subsequently treated for 2-3 hours at 80 C with paraformaldehyde vapor. The ganglia were vacuum infiltrated with Maraglas which was then polymerized at 60 C overnight. Serial sections 1 micron in thickness, were cut, examined on a Zeiss fluorescence microscope, and individual SIF cells or SIF cell clusters were identified.

In order to further characterize the morphology of the SIF cells and to be able to recognize them in plastic embedded sections prepared for electron microscopy, the sections containing SIF cells identified in the fluorescence microscope were stained with toluidine blue; the sections were then reexamined using bright field microscopy and the morphological characteristics of the identified SIF cells, stained with toluidine blue, were studied.

Because of the sparse distribution of SIF cells in these ganglia, serial sections, 1 micron in thickness, of the plastic embedded tissue were stained with toluidine blue and light microscopically examined for SIF cells. When cells or clusters were identified, thin sections for electron microscopy were taken. In this manner, it was possible to locate SIF cells and to study their ultrastructural characteristics. The tissue was examined and photographed using Phillips 200,300, and Zeiss 9S

electron microscopes.

Major Findings:

As indicated by their name, small intensely fluorescent (SIF) cells are characterized by their very bright fluorescence. These cells were relatively infrequent in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. They usually occurred in small clusters containing 3-15 cells. The SIF cell clusters were randomly distributed throughout the ganglia and were usually located in the vicinity of blood vessels. Fluorescent processes were not observed emanating from any individual SIF cells or from SIF cell clusters. The morphology of these cells was characterized further by staining with toluidine blue. When stained in this manner and viewed with normal bright field microscopy, the cells were found to be small, 10-20 microns in diameter, rounded or ovoid, with peripherally located aggregates of heterochromatin, and granular cytoplasm.

Due to the morphological similarities of ganglionic SIF cells and adrenal chromaffin cells, the question arose whether SIF cells are indeed chromaffin cells. Chromaffin cells in the adrenal gland are characterized by a positive chromaffin reaction. We therefore employed this reaction on sympathetic ganglia to determine if SIF cells are chromaffin positive. We found that SIF cells in sympathetic ganglia stain positively with the chromaffin reaction, indicating that they are indeed chromaffin cells.

Electron microscopic observation of the SIF-chromaffin cells reveals that they contain an abundance of large electron-dense granules. On the basis of the appearance of the granules, two cell types can be distinguished; one with very electron-dense granules, and the other with granules of moderate density. Additionally, axon-like cytoplasmic processes were not observed emerging from any chromaffin cells. Furthermore, no processes of chromaffin cells were seen penetrating the connective tissue capsule surrounding the clusters of chromaffin cells. In addition, chromaffin cells in the adrenal gland were also examined and appeared virtually identical to the SIF-chromaffin cells found in the sympathetic ganglia.

Significance to Bio-medical Research:

In the present investigation, we studied the distribution and morphology of SIF cells in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog in relation to their functional significance. Morphologically and histochemically the SIF cells appeared identical to chromaffin cells in the

adrenal glands. These observations suggest that SIF cells represent extra-adrenal chromaffin tissue located in sympathetic ganglia. In the present study, we are unable to find morphological evidence that SIF cells in bullfrog sympathetic ganglia make synaptic connections with the neurons. The SIF-chromaffin cells did not have the morphological characteristics of interneurons and thus would not function as adrenergic interneurons in synaptic pathways. This is in accord with physiological data the fast and slow synaptic potentials are due to cholinergic innervation of the neurons.

Proposed Course:

The continuation of the project will be directed toward further elucidation of the catecholamines contained in SIF-chromaffin cells and whether they play a role in modulating synaptic transmission in sympathetic ganglia or have other effects on neuronal function.

Publications:

Weight, F.F., and Weitsen, H.A.: Identification of small intensely fluorescent (SIF) cells as chromaffin cells in bullfrog sympathetic ganglia. Brain Res. 128: 213-226, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01307-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Electrical Excitability of Neuronal Membranes and the Actions of Calcium and Theophylline.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D.,Chief, Section on Synaptic Pharmacology	SMRN NIMH
Other:	N. A. Busis	M.D.	
	C. Swenberg	Ph.D.	SMRN NIMH
	J. Nash		SMRN NIMH

COOPERATING UNITS (if any)
None

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The electrical excitability of neuronal membrane was studied using the sucrose gap technique to investigate the sympathetic ganglion of the bullfrog. Action potentials were elicited in three ways: by antidromic stimulation across the sucrose gap; and by orthodromic stimulation through two separate presynaptic pathways. The effects of calcium ions and theophylline were studied on the electrogenic processes involved in action potential generation. We found that the spike after-hyperpolarization in these neurons has a calcium-sensitive component. The calcium sensitive component was found to be an increase in potassium permeability. Furthermore, this calcium-sensitive potassium conductance is potentiated by theophylline, a drug known to affect cellular calcium metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

In many neurons, calcium as well as sodium enters during the action potential. In previous studies, intracellular injection of calcium has been shown in several types of neurons to increase membrane permeability to potassium, and voltage clamp experiments have demonstrated that inward calcium currents can activate outward potassium currents. The action potential in many nerve cells is followed by a slower after-hyperpolarization, which results from increases of one or more potassium conductances. Therefore, calcium influx during an action potential might activate part or all of the subsequent after-hyperpolarization. The objective in this study was to investigate whether sympathetic neurons have a calcium-sensitive component involved in the electrogenesis of the spike after-hyperpolarization. In addition, the action of theophylline, a drug known to affect cellular calcium metabolism, was studied on the spike after-hyperpolarization.

Methods:

The experiments were conducted on the ninth and tenth paravertebral sympathetic ganglia of the bullfrog by means of the sucrose gap recording technique. The ganglia were continuously superfused with an oxygenated Ringer's solution. In experiments studying the effect of calcium removal on neuronal electrogenic processes, calcium-free Ringer's solution was prepared by omission of calcium chloride. The action of theophylline on electrogenic processes was studied by superfusion in the Ringer's solution using a concentration of 5 mM.

Major Findings:

The action potential after-hyperpolarization in sympathetic neurons was considerably and reversably potentiated by theophylline. The after-hyperpolarization was increased whether the action potentials were generated by othodromic B or C fiber stimulation or by antidromic stimulation of the neurons. The predominant effect of theophylline was an enhancement of the after-hyperpolarization duration. It increased the half-time for decay of the antidromically elicited after-hyperpolarization up to 800%. The duration of the potentiated after-hyperpolarization was inversely dependent on stimulus frequency, with maximal duration observed only when stimuli were administered more than one minute apart. Theophylline enhanced the after-hyperpolarization amplitude to a lesser extent, only increasing the peak amplitude of the antidromically elicited after-hyperpolarization up to 150%. Theophylline did not significantly affect the amplitude of the action potential elicited by otho-

dromically elicited action potential.

The action potential after-hyperpolarization in these sympathetic neurons has been shown previously to be generated by an increase in potassium conductance, and thus is decreased by hyperpolarizing current across the membrane. In this investigation, it was found that hyperpolarization of the resting membrane potential progressively reduced and abolished the theophylline potentiated after-hyperpolarization, as it does the normal after-hyperpolarization. These data indicate that the potassium conductance during the after-hyperpolarization is prolonged by theophylline. In addition, these data distinguish the theophylline potentiated after-hyperpolarization from the slow IPSP, which is potentiated by moderate hyperpolarizing current.

The potentiation of the spike after-hyperpolarization by theophylline was abolished by the removal of extracellular calcium ions. This was shown in two ways. First, the ganglia were superfused with a Ringer's solution containing normal calcium and theophylline until maximal after-hyperpolarization potentiation occured. Then they were superfused with Ringer containing theophylline but no calcium. In the absence of extracellular calcium, the after-hyperpolarization was markedly diminished in duration and amplitude, becoming even smaller than the pre-theophylline control. The potentiated after-hyperpolarization returned when calcium was restored. Second, ganglia were superfused with calcium-free Ringer for more than 4 hours. At that time, the addition of theophylline did not significantly alter the antidromically elicited after-hyperpolarization.

Significance to Bio-medical Research and the Program of the Institute:

Our data show that the repolarization phase of the action potential after-hyperpolarization in these neurons depends on extracellular calcium ions. It has been demonstrated previously that calcium as well as sodium ions can enter these neurons during the action potential. This suggest that calcium influx during the action potential activates a potassium conductance that prolongs the duration of the after-hyperpolarization. Alternatively, calcium influx may control the rate of inactivation of the after-hyperpolarization potassium conductance. In the present experiments, we also found that theophylline greatly potentiates the calcium-sensitive potassium conductance. Theophylline may increase stimulus-induced entry of calcium into these neurons, as it does in other tissues, thus augmenting the activation of the potassium conductance, however, we cannot exclude other possibilities, for example, that theophylline

enhances the calcium sensitivity of the after-hyperpolarization potassium conductance.

Theophylline inhibits cyclic nucleotide phosphodiesterases and potentiates cyclic nucleotide-dependent processes in many tissues. Cyclic nucleotides may be involved in cellular calcium metabolism and could play a role in the augmentation of the after-hyperpolarization by theophylline. In cardiac Purkinje fibers, theophylline and cyclic AMP increase both a slow inward current carried by calcium and sodium, and a slow outward carried predominantly by potassium.

Calcium-sensitive potassium conductances modulate the excitability and repetitive firing of neurons. Theophylline may therefore prove a useful tool for investigating regulation of neuronal activity and responsiveness.

Proposed Course:

The continuation of this project will be directed toward a further elucidation of how calcium ions and theophylline interact to control and regulate electrogenic processes in neurons.

Publications:

Neil A. Busis and Forrest F. Weight, Spike after-hyperpolarization of a sympathetic neuron is calcium-sensitive and is potentiated by theophylline. Nature, 263: 434-436, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01308-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Synaptic Transmission: Studies on the Physiology and Pharmacology of Postsynaptic Potential Generation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
	Peter A. Smith	Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)

None

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Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ionic mechanisms involved in the generation of several types of postsynaptic potentials were investigated in the sympathetic ganglia of bullfrogs, by intracellular and sucrose gap recording techniques. The fast EPSP has a marked decreased in membrane resistance at its peak and has a reversal potential at -16 mV. These data, together with ion change experiments, indicate that the fast EPSP is generated by an increase membrane conductance to both sodium and potassium ions. In contrast to this increased conductance mechanism, the slow postsynaptic potentials (the slow EPSP and the slow IPSP) are generated by different membrane mechanisms. Membrane resistance is increased during the slow EPSP, and membrane polarization shows that the slow EPSP reverses near the potassium equilibrium potential. We propose that the slow EPSP, in contrast to classical fast EPSPs, is generated by an inactivation of resting potassium conductance. The slow IPSP in these neurons is also associated with an increase in membrane resistance, and electrical polarization of the membrane indicates that the slow IPSP is nullified by a strong depolarizing current. We propose that the slow IPSP, in contrast with other types of fast IPSPs in the nervous system, is generated by an inactivation of resting sodium conductance.

Project Description:

Objectives:

Previous investigations in the vertebrate and invertebrate nervous system have shown that postsynaptic potential generation is due to an increase in ion conductance at virtually all chemically transmitting junctions which have been investigated. In sympathetic ganglia, slow postsynaptic potentials have been found with mechanisms which differ from those of other types of synaptic potentials in the central and peripheral nervous system. The objective of this study is to investigate the ionic membrane mechanism involved in the generation of these slow synaptic potentials, and their functional significance.

Methods:

The ninth and tenth paravertebral sympathetic ganglia of the bullfrog were studied in vitro with continuous perfusion of oxygenated Ringer's solution. Intracellular records were taken from sympathetic ganglion cells using microelectrodes filled with 3 M KCl. Standard techniques of electrophysiological recording were used, current being injected through the recording microelectrode by means of a Wheatstone bridge circuit. Preganglionic afferents were stimulated in the sympathetic chain and the 8th spinal nerve. Ganglion cells were identified on the basis of antidromic conduction velocity.

Major Findings:

The fast EPSP, like most other EPSPs, was found to be due to an increase ion conductance. Hyperpolarization of the membrane increased fast EPSP amplitude, and depolarization decreased its size. Further depolarization then reversed the fast EPSP to a hyperpolarizing potential. The reversal potential was approximately -16 millivolts.

The electrophysiological properties of the slow EPSP differed significantly from those of the fast EPSP. Membrane resistance, measured by constant current pulses, increased significantly during the slow EPSP, indicating a decrease in membrane conductance. In contrast to the effect of membrane polarization on the fast EPSP, depolarizing current increased the size of the slow EPSP, whereas hyperpolarizing current decreased slow EPSP amplitude. Further hyperpolarization of the membrane reversed the slow EPSP to a hyperpolarizing potential. The reversal potential for the slow EPSP was near the potassium equilibrium potential.

The electrophysiological properties of the slow IPSP also differed significantly from the properties of synaptic inhibition at most synaptic junctions. Membrane resistance tested during the slow IPSP by constant current pulses indicate

that membrane resistance increased during the slow IPSP. The effect of membrane polarization also differed from the effect of polarization on increased conductance IPSPs. Moderate hyperpolarizing current increased the amplitude of of the slow IPSP, whereas moderate depolarizing current decreased slow IPSP amplitude. Progressively stronger depolarizing current further decreased and then nullified the slow IPSP.

Significance to Bio-medical Research and the Program of the Institute:

The data in this investigation indicate that the fast EPSP, which is generated by the synaptic activation of nicotinic receptors, is due to an increase in sodium and potassium conductance of the membrane. This is similar to the mechanism of generation of most EPSPs in the vertebrate and invertebrate nervous system. On the basis of the data on the slow EPSP, we propose that the slow EPSP is generated by a decrease or inactivation of resting potassium conductance. This hypothesis provides an adequate explanation of the electrophysiologic properties of the slow EPSP demonstrated in this investigation. Similarly, to explain the properties of the slow IPSP found in this investigation, we propose that the slow IPSP is generated by a decrease or inactivation of resting sodium conductance. There are a number of suggestions that similar synaptic mechanisms are involved in slow postsynaptic potentials in the central nervous system; however, because of various technical and experimental limitations those mechanisms have not been adequately characterized. Thus, these mechanisms of synaptically inactivating resting membrane conductances may be of general significance in the synaptic control of neuronal function.

Proposed Course:

The continuation of this project will be directed toward further elucidation of how the action of a synaptic transmitter on postsynaptic membrane receptors leads to an inactivation of different membrane conductances. Attempts will also be made to determine whether these membrane changes are initiated by the synaptic activation of intracellular molecular changes, and if so, we will attempt to determine the molecular mechanisms involved. Two papers on this investigation are in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01309-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Long-lasting Control of Postsynaptic Membrane Permeability and its Role in Controlling Synaptic Transmission Efficacy.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D.,Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: J. A. Schulman

COOPERATING UNITS (if any)

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Laboratory of Neuropharmacology

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Slow decreases of ionic conductance across neuronal cell membranes, which generate slow postsynaptic potentials, can increase the effectiveness of synaptic transmission. Slow conductance decreases of sufficient magnitude increase the amplitude of monosynaptic fast EPSPs in B cells of the bullfrog sympathetic ganglia. By this postsynaptic mechanism, activation of one synaptic pathway can cause an increase in transmission, lasting several minutes, across another synapse. This may provide an important mechanism for synaptic integration and control of neuronal interaction.

Project Description:

Objectives:

An understanding of the mechanisms of synaptic transmission and their regulation may be important in the elucidation of the cellular mechanism underlying complex nervous function. The postsynaptic mechanisms by which EPSPs are generated and combined might provide a locus for control of synaptic function. However, the vast majority of known postsynaptic electrical responses are of short duration (tens of msec). Thus, postsynaptic interactions of such responses are necessarily brief and require a high degree of synchrony of convergent inputs. These interactions, therefore, do not provide a lasting modification of responsiveness. In addition, when fast synaptic potentials combine, the result is at most the algrebraic sum, and often is less. The object of the present experiments was to test the effect of synaptically activated conductance decreases on the effectiveness of synaptic transmission. In this way, we evaluated the role of postsynaptic membrane permeability in the control of synaptic function.

Methods Employed:

Synaptic potentials were recorded intracellularly in the ninth and tenth paravertebral sympathetic ganglia of bullfrogs, in vitro. Preganglionic axons were stimulated in the sympathetic chain and the eighth spinal nerve. Iontophoresis was used to administer methacholine directly to the neuron under investigation. Standard electrophysiological techniques were used, a bridge circuit being utilized to inject current intercellularly. The late slow EPSP was elicited by stimulating the eighth spinal nerve at a frequency of 50 Hz for 2 seconds.

Major Findings:

The slow EPSP, which is generated by a decrease in membrane potassium conductance, was mimicked by the iontophoretic administration of methacholine. The effect of this conductance decrease on synaptic transmission was studied by observing the effect of iontophoretically administered methacholine on fast EPSPs. It was found that fast EPSP amplitude increased with methacholine activated increases in membrane resistance of greater than 8 megohms. The duration of the fast EPSP also increased during the methacholine induced decrease in membrane conductance. In addition, these enlarged EPSPs often activated the postsynaptic generation of action potentials.

It is important to know whether such a postsynaptic potentiation of synaptic transmission can be elicited by stimulation

of physiological pathways. To test this possibility, we utilized a separate preganglionic pathway to generate a conductance decrease. Repetitive stimulation of the eighth spinal nerve produces a slow depolarization lasting many minutes, the late slow EPSP. Membrane conductance was found to be decreased during the late slow EPSP. As with the methacholine response, the amplitude of the fast EPSP increased during the late slow EPSP, and the increased amplitude of the fast EPSPs often initiated action potential generation. The increase in postsynaptic action potential generation by fast EPSPs could last for 2 or more minutes. For example, in one experiment, at the peak of the response, 5 out of 6 fast EPSPs elicited action potentials, compared to none in a control. This is a clear demonstration of the facilitation of transmission in one synaptic pathway by activation of another.

Significance to Bio-medical Research and the Program of the Institute:

This investigation indicates that different types of depolarization and membrane permeability changes have different consequences for neuronal interaction and synaptic transmission. That is, conductance increases and electrical depolarization of the membrane decrease synaptic potentials generated by a given excitatory input, whereas conductance decreases potentiate them. By augmenting, rather than diminishing, a synaptic input, a conductance decrease makes that input more effective than generation of action potentials.

This control of the efficacy of synaptic transmission may be important in the function of neurons of the central nervous system. Central neurons receive an abundance of converging inputs, and several types of central neurons have conductance-decrease responses. It is thus possible that increasing transmission in a synaptic pathway by a conductance-decrease response may have a function in information processing in the central nervous system. Further, lasting modification of responsiveness to a synaptic input might be a mechanism for storage of information at the cellular level. Long-term control of postsynaptic membrane properties might provide such a modification of responsiveness.

Proposed Course:

The continuation of this project will be directed toward a further elucidation of how changes in membrane permeability control the efficacy of synaptic transmission, and whether lasting changes in resting membrane permeability can be activated by periods of synaptic stimulation. In addition, we shall also

attempt to determine the molecular mechanisms involved in such long-lasting changes in membrane permeability.

Publications:

Schulman, J.A. and Weight, F. F.: Synaptic transmission: long-lasting potentiation by a postsynaptic mechanism. Science, 194: 1437-1439, 1976.

Weight, F.F. and Schulman, J.A.: Long duration potentiation of synaptic transmission by synaptically and iontophoretically activated decreases in postsynaptic membrane conductance. In Symposium on "Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System", in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01310-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Biochemistry and Pharmacology of Cyclic Guanosine Monophosphate (Cyclic GMP) and Cyclic Adenosine Monophosphate (Cyclic AMP) Activation by Synaptic Stimulation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other: P. Greengard
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COOPERATING UNITS (if any)
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Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The stimulation of cholinergic preganglionic nerve fibers resulted in an increase in cyclic guanosine monophosphate (Cyclic GMP) and cyclic adenosine monophosphate (Cyclic AMP) in the sympathetic ganglia of the bullfrog. When the release of synaptic transmitter was prevented by a high-magnesium/low-calcium Ringer's solution, stimulation of preganglionic nerve fibers did not increase either cyclic GMP or cyclic AMP content. Atropine, a muscarinic antagonist that blocks the generation of both the slow IPSP and the slow EPSP in this tissue, also blocked the increase in cyclic GMP caused by preganglionic stimulation, but not the increase in cyclic AMP. The data indicate that the increases in both cyclic GMP and cyclic AMP are associated with synaptic transmission and are consistent with the possibility that cyclic GMP may be involved in the postsynaptic action of acetylcholine at muscarinic cholinergic synapses.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Cyclic GMP is distributed in a wide variety of tissues, but little is known about its functional role. The functional significance of cyclic GMP in the nervous system and particularly the possible relationship of this nucleotide to cholinergic transmission require further clarification. The sympathetic ganglion is well suited for an investigation of a possible role of cyclic GMP in cholinergic transmission, because the cholinergic preganglionic nerve fibers form synapses with the neurons in the ganglion. Furthermore, in the bullfrog sympathetic ganglion, muscarinic cholinergic mechanisms are involved in the generation of both the slow EPSP and the slow IPSP. Additionally, data are available on the ionic basis of these slow postsynaptic potentials. We therefore used these sympathetic ganglia to investigate whether cyclic nucleotide content can be altered by stimulation of the preganglionic nerve fibers.

Methods Employed:

The ninth and tenth paravertebral sympathetic ganglia were excised from bullfrogs and placed in a bath of oxygenated Ringer's solution, together with the corresponding ganglia from the contralateral side, which served as the unstimulated control. The preganglionic nerve fibers were stimulated at a frequency of 10 Hz for various periods of time, the postganglionic response being monitored throughout the period of stimulation. Immediately after stimulation, the stimulated ganglia were immersed in a boiling water bath for 3 minutes. The cyclic nucleotides content of the ganglia was subsequently determined by radioimmunoassay.

Major Findings:

Stimulation of preganglionic nerve fibers produced a significant increase in cyclic GMP content. Cyclic GMP was elevated by a period of stimulation as short as 6 seconds. The increase in cyclic GMP was rapid during the first 15 to 30 seconds of stimulation, reaching a maximum concentration approximately twice that of control. This elevated cyclic GMP content was maintained for at least 2 minutes, the longest period of stimulation tested. Cyclic AMP content was also increased by preganglionic stimulation; for example, stimulation for 2 minutes increased the cyclic AMP content to more than 700% of control.

The increase in cyclic GMP and cyclic AMP content that occurred with 2 minutes of stimulation in normal Ringer's solution was not observed in ganglia stimulated for the same period of time in a high-magnesium, low-calcium Ringer's solution. Since high-magnesium, low-calcium Ringer is known to prevent transmitter release from nerve endings in the sympathetic

ganglion, this result suggests that the increase in cyclic nucleotide content in the sympathetic ganglia is associated with synaptic transmission, rather than with impulse conduction along the preganglionic nerve fibers.

Since atropine is a competitive antagonist of acetylcholine at muscarinic postsynaptic receptors, and blocks the generation of both the slow IPSP and the slow EPSP in sympathetic ganglia, we used atropine to determine whether muscarinic receptors might be involved in the increase in cyclic nucleotide content following stimulation of preganglionic nerve fibers. In the presence of atropine, preganglionic stimulation did not produce a significant increase in cyclic GMP content. On the other hand, the increase of cyclic AMP was not significantly reduced in the presence of atropine.

Significance to Bio-medical Research and the Program of the Institute:

Previous investigations have shown that cyclic GMP in nervous tissues can be elevated by the administration of acetylcholine or muscarinic cholinergic agonist, but it has not been previously demonstrated that stimulation of synaptic pathways can change cyclic GMP content. The data in this study demonstrates that cyclic GMP content of nervous tissue can be significantly increased by stimulation of presynaptic cholinergic axons. The increase in cyclic GMP and in cyclic AMP was antagonized by high-magnesium, low-calcium concentration suggesting that the increase is associated with synaptic transmission. The selective antagonism by atropine of the increase in cyclic GMP suggests that the cyclic GMP increase involves the activation of muscarinic postsynaptic receptors. The data in the present investigation are consistent with the possibility that cyclic GMP may be involved in the postsynaptic action of acetylcholine of muscarinic cholinergic synapses. Further investigation is necessary to determine the postsynaptic receptors involved in the cyclic AMP activation.

Proposed Course:

The continuation of the project will be directed toward a further elucidation of the role of cyclic nucleotides in synaptic function in nervous tissue. A paper on this investigation is in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01311-01 SMRN

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Investigation of the Possible Involvement of Cyclic Nucleotides in Synaptic Potential Generation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
Others:	N. A. Busis	M.D.	
	P. A. Smith	Ph.D.	SMRN NIMH
	C. Swenberg	Ph.D.	SMRN NIMH
	J. Nash		SMRN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The possibility that the cyclic nucleotides, cyclic AMP and cyclic GMP may mediate the generation of the slow synaptic potentials was investigated in sympathetic ganglia of the bullfrog, using the sucrose gap technique. The administration of cyclic GMP, cyclic AMP, and their derivatives had no significant effect on membrane potential. The administration of theophylline (5 mM), an inhibitor of phosphodiesterase - the catabolic enzyme for cyclic nucleotides, produced a depolarization of the membrane and an increase in membrane conductance. The muscarinic slow IPSP and the hyperpolarization produced by the administration of methacholine were potentiated by theophylline. The muscarinic slow EPSP and the depolarization produced by the administration of methacholine were reduced by theophylline. The noncholinergic late-slow EPSP was reduced by theophylline. In calcium-free Ringer, theophylline greatly enhanced the methacholine hyperpolarization; in addition, the methacholine depolarization was virtually abolished. These results raise the question of whether the cyclic nucleotides may have a functional role other than generation of slow postsynaptic potentials.

Project Description:

Objectives:

In vertebrate sympathetic ganglia stimulation of preganglionic afferents evokes long-duration postsynaptic potentials, the slow IPSP and the slow EPSP. The mechanism of generation of these slow postsynaptic potentials is of considerable interest because these synaptic potentials are not associated with increases in membrane conductance, as occurs in the generation of most fast postsynaptic potentials, and their prolonged duration allows novel long-lasting mechanisms of synaptic integration and control of neuronal interaction. Additionally, postsynaptic potentials with apparently similar mechanisms of generation have been found in the vertebrate central nervous system.

Cyclic nucleotides have been proposed previously to function as intracellular second messengers mediating the generation of the slow postsynaptic potentials in sympathetic ganglia; the slow IPSP being mediated by cyclic AMP and the slow EPSP by cyclic GMP. This hypothesis is based on certain electrophysiological and bio- chemical evidence from rabbit and bovine sympathetic ganglia. The electrophysiological evidence from rabbit ganglia includes the alteration of resting membrane potential by the extracellular administration of cyclic nucleotides and the potentiation of the slow IPSP by theophylline, an inhibitor of cyclic nucleotides phosphodiesterase that potentiates cyclic nucleotide dependent processes in many tissues.

The membrane mechanisms involved in the generation of both the slow EPSP and the slow IPSP have been most extensively investigated by electrophysiological techniques in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. In biochemical studies of bullfrog ganglia, stimulation of the preganglionic afferents have been found to result in an increase in both cyclic GMP and cyclic AMP, suggesting the possibility of cyclic nucleotide involvement in the generation of the slow postsynpatic potentials. To compliment these biochemical studies and to further extend electrophysiological investigations into the nature of slow potential generation, we have studied the effect of the administration of cyclic nucleotides and theophylline to these sympathetic ganglia.

Methods Employed:

The ninth or tenth paravertebral sympathetic ganglia of bullfrogs were excised and placed in a special plexiglas sucrose gap apparatus. The ganglion was continuously superfused with oxygenated Ringer's solution and the preganglionic

nerves in the sympathetic chain and the eighth spinal nerve were placed in a pool of liquid paraffin for stimulation. Recording was accomplished with two calomel electrodes, isotonic KCl. Standard electrophysiological techniques were used to record the potentials from the sucrose gap chambers.

Preganglionic stimulation was accomplished by delivering brief current pulses from isolated stimulators through bipolar platinum electrodes placed on the preganglionic afferents in the paraffin pool. The slow EPSP was elicited by repetitive stimulation of the sympathetic chain rostral to the seventh ganglion. The slow IPSP and the late slow EPSP were elicited by repetitive stimulation of the eighth spinal nerve.

Calcium-free Ringer's solution was prepared by omission of calcium chloride from the standard Ringer's solution. In most experiments either nicotine (30 uM) or d-tubocurarine 70 M) were used to antagonize the fast EPSP and the nicotinic cholinergic actions of methacholine.

Major Findings:

The slow IPSP in the sympathetic ganglia was considerably and reversably potentiated by theophylline. The slow IPSP was increased whether it was generated in the presence of nicotine or d-tubocurarine block. The predominant effect of theophylline was an enhancement of slow IPSP amplitude. Theophylline also increased the duration of the slow IPSP but to a lesser extent. The slow EPSP was also affected by theophylline; however, the predominant effect was a reduction in slow EPSP amplitude.

Repetitive stimulation of the eighth spinal nerve also elicits a slow depolarization in these ganglia, the late slow EPSP. Although the late slow EPSP is abolished by the removal of extracellular calcium ions, it is not blocked by atropine, nicotine, or d-tubocurarine, and thus is considered to be noncholinergic. In virtually all experiments, the late slow EPSP was reduced in amplitude by theophylline.

Theophylline may potentiate transmitter release from preganglionic nerve terminals and thus affect the amplitude of slow postsynaptic potentials. To control for this possibility, we use brief superfusions of the muscarinic agonist, methacholine (1mM), to mimic the generation of the slow IPSP and the slow EPSP. The methacholine hyperpolarization was considerably and reversably potentiated by theophylline, just as the slow IPSP was potentiated. The methacholine depolarization was

affected similarly by theophylline, as compared to the slow EPSP. In most experiments, theophylline reversably reduced the amplitude of the methacholine depolarization.

In a separate investigation, we found that theophylline potentiates the spike after-hyperpolarization in these sympathetic neurons, and that this potentiation is abolished by the removal of extracellular calcium ions. We therefore studied whether theophylline could alter the methacholine response of these neurons in calcium-free Ringer. Since synaptic transmission is abolished in the absence of extracellular calcium ions, this part of the study was conducted on the methacholine responses. In the absence of extracellular calcium ions, theophylline resulted in a very large potentiation of the methacholine hyperpolarization. Furthermore, the methacholine depolarization was virtually abolished.

In addition to the effects of theophylline on the slow postsynaptic potentials, theophylline also produced a depolarization of membrane potential. This depolarization achieved a plateau in 30-60 minutes after the introduction of the drug and persisted as long as theophylline was superfused. Return to the theophylline-free Ringer was associated with a gradual repolarization over 1 hour or more. Theophylline depolarized ganglia whether they were superfused with normal Ringer or with Ringer containing nicotine or d-tubocurarine. In addition to the depolarization, the recorded base-line during theophylline became irregular, which may be related to spontaneous hyperpolarizations seen in individual neurons with intracellular recording. The theophylline depolarization was associated with an increase in membrane conductance.

Cyclic GMP and several derivatives (dibutyryl cyclic GMP, 8- benzylthio cyclic GMP, and parachlorophenylthio cyclic GMP), as well as cyclic AMP and derivatives (dibutyryl cyclic AMP and monobutyryl cyclic AMP) superfused at concentrations of 1 mM for one to several minutes had no significant effect on the resting membrane potential of these sympathetic neurons, whether these substances were superfused with normal Ringer, or with Ringer nicotine, d-tubocurarine or theophylline. These results are in agreement with other experiments in this laboratory, using intracellular recording, in which bath applications or iontophoresis of these cyclic nucleotides or their derivatives had no significant effect on membrane potential.

Significance to Bio-medical Research and the Program of The Institute:

Theophylline is well known as an inhibitor of cyclic nucleotide phosphodiesterases, and it has been used as a pharmacological probe for the involvement of cyclic nucleotides in a variety of cellular processes. In the present investigation, we have found that theophylline affects slow postsynaptic potential generation, muscarinic postsynaptic responses, resting membrane potential, and membrane conductance. The elucidation of the mechanisms of these actions and their interrelation with one another and with inhibition of ganglionic cyclic nucleotide phosphodiesterase may provide insight into the mechanism of slow synaptic potential generation and the role of cyclic nucleotides in neuronal function.

Theophylline potentiates cyclic nucleotide-dependent processes in many tissues. If production of the slow IPSP and the slow EPSP were each mediated by cyclic nucleotides then one might expect theophylline to potentiate both responses. Our data show that theophylline potentiated only the slow IPSP. Since theophylline has been found to potentiate the release of acetylcholine at the neuromuscular junction, such a presynaptic effect could account for the potentiation of slow IPSP. The potentiation of the methacholine hyperpolarization by theophylline in these experiments indicates that theophylline can potentiate muscarinic hyperpolarization by postsynaptic mechanisms. Separate investigations in this laboratory have demonstrated that preganglionic stimulation increases both cyclic GMP and cyclic AMP in these neurons, suggesting that these cyclic nucleotides might be involved in the generation of synaptic potentials. The potentiation of the slow IPSP observed in the present experiments by theophylline may be due to inhibition of phosphodiesterase, although other interpretations are also possible.

The effect of theophylline on the muscurinic slow EPSP and the methacholine depolarization in the present experiments was in most cases a reduction in the amplitude of the depolarization. These data do not support the possibility that the slow EPSP is mediated by cyclic GMP. It should be noted, however, that the data also do not exclude a mediation by a cyclic nucleotide, since it is possible that another theophylline effect, such as the increase in membrane conductance, may prevent a potentiation from being observed.

In addition to inhibiting phosphodesterase, theophylline can also affect cellular calcium metabolism. In a separate investigation in this laboratory, we have demonstrated a calcium sensitive component in the spike after-hyperpolarization and

showed that this calcium-sensitive potassium conductance is potentiated by theophylline. Since theophylline potentiates both the spike after-hyperpolarization and the slow IPSP, it is of interest to compare these two hyperpolarizing potentials. The action potential after-hyperpolarization is due to an increase potassium conductance, and thus is decreased by hyperpolarizing currents. On the other hand, the slow IPSP is generated by a decrease in sodium conductance and thus is potentiated by moderate hyperpolarizing current. Additionally, the theophylline-induced potentiation of the after-hyperpolarization is abolished by the removal of extracellular calcium. In contrast, the theophylline-induced potentiation of the methacholine hyperpolarization is not abolished by calcium-free Ringer, and, in fact, this muscarinic hyperpolarization is potentiated. The possibility should be considered that the theophylline induced effects on slow synaptic potential generation may be due to effects of theophylline on cellular calcium metabolisms or other membrane actions.

In the present study, the administration of cyclic GMP, cyclic AMP, and several of their derivatives failed to demonstrate any consistent effect on resting membrane potential. These negative findings should be interpreted with caution, however, due to lack of knowledge of the uptake and metabolism of exogenous cyclic nucleotides in these tissues. Nevertheless, these results raise the question of whether the increase in cyclic nucleotides produced by stimulation of presynaptic nerve fibers may play a role other than the generation of slow postsynaptic potentials.

Proposed Course:

The continuation of this project will be directed toward the elucidation of the functional role of cyclic nucleotides in nervous tissue. A paper on this investigation is in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01312-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)

Physiology and Pharmacology of Electrogenic Sodium Pump Activation and Inhibition in Neurons.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Peter A. Smith Ph.D. SMRN NIMH

Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology. SMRN NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The physiology and pharmacology of the activation and inhibition of the electrogenic sodium pump was investigated in the sympathetic ganglia of bullfrogs, by the sucrose gap technique. The administration of nicotinic cholinergic agonists produces a brief depolarization that is followed by an after-hyperpolarization that is due to activation of the electrogenic sodium pump. This electrogenic pumping of sodium was rapidly inhibited by potassium-free Ringer. The after-hyperpolarization was also inhibited after one hour in ouabain (1 μM). Potassium-free Ringer resulted in an accumulation of intracellular sodium ions, so that return to normal Ringer solution resulted in hyperpolarization of the membrane due to activation of the electrogenic sodium pump. This hyperpolarization was also inhibited after one hour in ouabain (1 μM). These results indicate that the electrogenic sodium pump can be activated in two ways: following a nicotinic cholinergic depolarization; and by return from potassium-free Ringer to normal Ringer's solution. Furthermore, the electrogenic sodium pump can be inhibited by potassium-free Ringer and by ouabain.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

In many, if no all, neurons, the outward pumping of sodium is coupled to the inward transport of potassium, with more sodium extruded than potassium taken in, thus producing an outward movement of positive charge. This electrogenic pumping of sodium produces a hyperpolarization of the membrane, without a change in membrane permeability. The objective of the present experiments was to characterize the activation of the electrogenic sodium pump and its inhibition.

Methods:

The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglia of the bullfrog using the sucrose gap technique. The preparation was continuously superperfused with an oxygenated Ringer's solution containing a potassium concentration of 2 mM. In experiments using potassium-free Ringer, KCl was omitted without change of the other ions. Drugs were added to the superperfusing Ringer's solution.

Major Findings:

The administration of acetylcholine or other nicotinic cholinergic agonists produces a brief depolarization due to an increased conductance to sodium and potassium ions. This is followed by an after-hyperpolarization that results from the electrogenic extrusion of accumulated intracellular sodium ions. This nicotinic afterhyperpolarization was used to characterize inhibition of electrogenic sodium pump by ouabain and potassium-free Ringer. Potassium-free Ringer rapidly inhibited the after-hyperpolarization. The inhibition persisted as long as the preparation was bathed in the potassium-free Ringer, but it was rapidly reversible. Inhibition of the after-hyperpolarization by ouabain occurred more slowly. The after-hyperpolarization was abolished after one hour in ouabain (1 M). This effect was only slowly reversable with the restoration of the after-hyperpolarization taking 4 hrs or more. These results indicate that potassium-free Ringer and ouabain are effective inhibitors of the electrogenic sodium pump in this tissue.

In addition, following the return from potassium-free Ringer to normal Ringer's solution there was a hyperpolarization of the membrane. This hyperpolarization was also inhibited after one hour in ouabain (1 M) and this inhibition was also slowly reversable. This indicates that the hyperpolarization is

due to the electrogenic pumping of the sodium ions accumulated intracellularly during sodium pump inhibition with potassium-free Ringer.

Significance to Bio-medical Research and the Program of the Institute:

The electrogenic sodium pump is important in nervous tissue for maintaining ionic gradients necessary for the functioning of nerve cells. In this investigation, we demonstrated two means of activating the electrogenic sodium pump in order to study its properties and the ability of certain experimental procedures or pharmacologic agents to inhibit the pump. We have found that potassium-free Ringer and ouabain (1 M) function as effective inhibitors of the electrogenic pumping of sodium ions in this tissue. These data may be of importance in attempting to understand the functional significance of the electrogenic sodium pump in the nervous system.

Proposed Course:

The continuation of this project will be directed toward the further elucidation of the mechanisms involved in the activation of the electrogenic sodium pump and the functional significance of electrogenic sodium pumping in nervous tissue. A paper on this investigation is in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01313-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Pharmacology of Electrogenic Sodium Pump Inhibitors on the Generation of Slow Synaptic Inhibition.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Smith	Ph.D.	SMRN NIMH
	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH

COOPERATING UNITS (if any)

None

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SECTION
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NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the present experiments, the possibility of a direct role of the electrogenic sodium pump in slow synaptic inhibition was tested. The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglia of the bullfrog using the sucrose gap technique. We found that although electrogenic sodium pumping was inhibited by ouabain or potassium-free Ringer, the slow IPSP was not blocked by ouabain and was enhanced in potassium-free Ringer. These data are not consistent with the hypothesis that the slow IPSP results from an activation of the electrogenic sodium pump.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The mechanism of synaptic transmission at most synapses involves an increase in the conductance of the postsynaptic membrane for certain ions. Certain slow postsynaptic inhibitions in both the central and peripheral nervous systems are, however, not generated in this way. It has previously been proposed that the slow inhibitory postsynaptic potential (IPSP) in sympathetic ganglia, and certain slow postsynaptic inhibitions in the central nervous system, are due to a unique mechanism, the synaptic activation of the electrogenic sodium pump. In the present experiments, the direct role of the electrogenic sodium pump in slow postsynaptic inhibition was tested.

Methods Employed:

The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglia of the bullfrog using the sucrose gap technique. The preparation was continuously superperfused with an oxygenated Ringer's solution. In experiments using a potassium-free Ringer's solution, KCl was omitted without a change of the other ions. The slow IPSP was elicited by stimulating the eighth nerve at a frequency of 50 Hz for 2 seconds. Drugs were administered by addition to the superperfusing Ringer's solution.

Major Findings:

The application of potassium-free Ringer's solution produced little or no change in resting membrane potential, but resulted in a rapid and persistent increase in the amplitude of the slow IPSP. This increase in slow IPSP amplitude persisted as long as the preparation was perfused with potassium-free Ringer. To investigate the muscarinic slow IPSP, the nicotinic fast EPSP must be antagonized. When nicotine was used to antagonize the fast EPSP, 1 hour in ouabain (1 μM) resulted in a partial reduction in slow IPSP amplitude. When d-tubocurarine was used as a nicotinic antagonist, however, no significant reduction was observed after 1 hour in ouabain. Even after 90 minutes in ouabain, the administration of potassium-free Ringer still increased the amplitude of the slow IPSP.

Significance to Bio-medical Research and the Program of the Institute:

The present experiments provide information on the mechanism of generation of slow postsynaptic inhibition. It had been proposed that the slow IPSP is due to an activation of the electrogenic sodium pump. The present experiment show that although

potassium-free Ringer and ouabain inhibit the electrogenic pumping of sodium, they do not block the slow IPSP. This suggests that the slow IPSP is not due to the synaptic activation of the electrogenic sodium pump. Another hypothesis proposes that the slow IPSP is generated by a decrease in resting sodium conductance. The present results can be readily explained by and support this hypothesis. The mechanism of generation of the slow IPSP may be important for information processing in the nervous system.

Proposed Course:

The continuation of the project will be directed toward a further elucidation of the role of the electrogenic sodium pump in neuronal function.

Publications:

Smith, P.A. and Weight, F.F.: Role of electrogenic sodium pump in slow synaptic inhibition is re-evaluated. Nature, 267: 68-70, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01314-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Lithium: Physiology and Pharmacology of Effects on Neuronal Membrane and Synaptic Potential Generation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Smith	Ph.D.	SMRN NIMH
	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular mechanisms of the action of lithium in the nervous system is of interest because of its importance in treating certain types of mental illness. We tested the effect of lithium on the electrogenic sodium pump and the slow IPSP in bullfrog sympathetic ganglia, using the sucrose gap technique. The administration of acetylcholine (3 mM) produces a nicotinic depolarization followed by an after-hyperpolarization that results from the activation of the electrogenic sodium pump. When sodium in the Ringer's solution was replaced by lithium, the acetylcholine depolarization still occured but the after-hyperpolarization was abolished. Presumably lithium is not extuded by the electrogenic sodium pump, as has been found previously in other tissues. Lithium Ringer also blocked the muscarinic slow IPSP and the hyperpolarizing response to agonists such as methacholine. We have recently found that ouabain and potassium-free Ringer can effectively inhibit the electrogenic sodium pump without blocking the slow IPSP, indicating that the slow IPSP is not generated by activation of the electrogenic sodium pump. In view of this, we conclude that the effect of lithium on the slow IPSP is not a direct consequence of sodium pump inhibition.

Project Description:

Objectives:

The cellular mechanisms of action of lithium are of particular interest because of its importance in treating manic-depressive psychosis. The objective of the present experiments was to study the effect of lithium on membrane permeability, on the electrogenic sodium pump, and on various electrogenic processes in neuronal membrane, including the generation of synaptic potentials.

Methods Employed:

In this study, the effects of lithium were investigated on the ninth and tenth paravertebral sympathetic ganglia of the bullfrog, in vitro. The sucrose gap technique was used to record changes in membrane potential and the generation of electrogenic processes. The ganglion was continuously superfused with oxygenated Ringer's solution. The effect of lithium was investigated by substituting lithium ions for sodium ions in the Ringer's solution. Drugs were administered by addition to the superfusing Ringer's solution.

Major Findings:

The administration of lithium Ringer resulted in a hyperpolarization of the membrane potential of the sympathetic ganglion neurons. To determine the possible ionic basis of this membrane hyperpolarization, electrical resistance of the membrane was tested with constant current pulses. It was found that membrane resistance increased the lithium Ringer. This indicates that membrane conductance was decreased in the lithium Ringer, suggesting that lithium conductance is lower in these neurons than sodium conductance.

The effect of lithium was also tested on the generation of synaptic potentials. In addition to hyperpolarizing the membrane potential, lithium Ringer was found to markedly reduce or abolish the generation of the slow IPSP. Although there were some changes in the fast EPSP and the slow EPSP, both of these synaptic potentials were generated in the presence of the lithium Ringer's solution. Since the effects of lithium on the slow IPSP might be due to presynaptic actions of lithium, the effect of lithium was also tested on the hyperpolarization produced by the administration of the muscarinic agonist, methacholine, which directly activates muscarinic postsynaptic receptors to mimic the slow IPSP. As was found with the effect of lithium on the slow IPSP, lithium abolished the hyperpolarization produced by the administration of methacholine.

In addition to these cellular actions, lithium was also found to substitute extracellularly for potassium ions in activation of the electrogenic sodium pump. If the ganglia are treated with potassium-free Ringer's solution, there is a resultant accumulation of intracellular sodium ions. When extracellular potassium ions are restored by superfusion with a normal Ringer's solution, the electrogenic sodium pump is reactivated and a membrane hyperpolarization results due to the electrogenic pumping of sodium ions. The substitution of 15 mM lithium Ringer for potassium-free Ringer also results in a reactivation of the electrogenic sodium pump.

Significance to Bio-medical Research and the Program of the Institute:

In this investigation, lithium Ringer's solution has been found to produce a hyperpolarization of the neurons and a decrease or abolition of the slow IPSP and the methacholine hyperpolarization. The reduction in slow IPSP amplitude and the amplitude of the methacholine hyperpolarization would not appear to be due to the hyperpolarization of the membrane produced by lithium, because electrical hyperpolarization of the membrane results in a potentiation of both the slow IPSP and the methacholine hyperpolarization. In a separate investigation, we have found that ouabain and potassium-free Ringer can effectively inhibit the electrogenic sodium pump in these neurons, without blocking the slow IPSP or the methacholine hyperpolarization, indicating that the slow IPSP is not generated by activation of the electrogenic sodium pump. In view of this, we conclude that the effect of lithium on the slow IPSP is not consequence of sodium pump inhibition.

The administration of lithium Ringer produced not only a hyperpolarization of the neuronal membrane potential, but also an increase in membrane resistance. This indicates that membrane conductance was decreased in lithium Ringer and suggests that the resting permeability of the membrane to lithium is less than that to sodium ions. Since the slow IPSP is thought to be due to be generated by a decrease in resting sodium conductance, a low resting lithium conductance may account for the reduction in the amplitudes of the slow IPSP and methacholine hyperpolarization. On the other hand, it is known from previous investigations that lithium ions are not extruded by the electrogenic sodium pump. Therefore, it is also possible that the reduction in slow IPSP and methacholine hyperpolarization amplitudes may be due to an intracellular accumulation of lithium ions. Thus, the mechanism of the lithium inhibition of the slow IPSP is, at present, uncertain, and is currently the subject of active investigation.

Proposed Course:

The continuation of these project will be directed toward further elucidation of the mechanism and functional significance of lithium's action on the electrogenic processes in nervous tissue. A report on this investigation will be presented at the Neuroscience Meeting in November.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01315-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Electrophysiology & Cytology of Hippocampal Formation Transplants in the Anterior Chamber of the Eye.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Taylor	Ph.D.	SMRN NIMH
	Barry J. Hoffer	Ph.D.,M.D.,Dept. of Pharmacology, Univ.Colorado, Denver, Colorado.	
	Robert Freedman	M.D., Dept. Psychiatry, Univ. of Chicaco, Chicago, Ill.	

COOPERATING UNITS (if any)
Lars Olson and Ake Seiger, Karolinska Institute, Stockholm, Sweden.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Developmental Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pieces of fetal rat hippocampus transplanted to the anterior chamber of the eye became vascularized from the host iris and proliferate markedly, developing an adult organization in oculo. Cytological and electrophysiological studies revealed the development of the various strata normally associated with the adult hippocampus. Furthermore these studies revealed a marked chemosensitivity of pyramidal cells to acetylcholine via a muscarinic receptor, suggesting that normal pharmacological and cytological development of the hippocampus proceeds in the absence of extrahippocampal afferents. In addition, the in oculo hippocampus develops a marked propensity for epileptogenic activity, much like that reported in situ.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objective of the present study was to determine if the fetal rat hippocampus could develop in oculo and whether its development in the absence of afferent input proceeded in normal fashion. In addition, pharmacological sensitivity studies could be carried out to determine the extent to which the normal receptivity of the hippocampus pyramidal cells was altered by the transplantation technique.

Methods Employed:

Electrophysiological recordings were obtained from hippocampal transplants which had matured in oculo for approximately 1 month. Neurohumoral substances could administered to the transplant via one of the peripheral barrels of 5-barrelled micropipette by microiontophoresis. Compounds could also be administered by either perfusion into the anterior chamber of the eye or by parenteral injection into the host animal. Changes in electrical activity to summed drug responses could be determined using a PDP-12 computer (see project Z01 MH 01298-08). Cytological studies were carried out using cholinesterase staining and formaldehyde induced fluorescence to demonstrate the presence of cholinergic and noradrenergic nerve fibers in the transplants.

Major Findings:

Pieces of fetal rat hippocampus which have been transplanted to the anterior chamber of the eye became vascularized, proliferated, matured and developed an adult organization in oculo. Cytological studies revealed the various striata associated with the adult hippocampus, while cholinesterase staining suggested that such transplants were invaded by cholinergic nerve fibers derived from the autonomic ground plexus of the host iris. Similarily, formaldehyde-induced fluorescence revealed the presence of noradrenergic nerve fibers within the transplant which were also derived from the host iris.

Electrical stimulation of the transplant surface elicited negative and positive field potentials at various depths in the transplants which could be associated with excitation and inhibition of the pyramidal cells. The responses of these pyramidal cells to the microiontophoretic application of substances revealed a muscarinic cholinergic excitatory action of acetylcholine much like that reported in situ. This excitatory response to acetylcholine was closely mimicked by cyclic 3',5'-(guanosine) monophosphate (cyclic GMP). Responses to both acetylcholine and cyclic GMP could be antagonized by atropine

and potentiated by phosphodiesterase inhibitors, suggesting that cyclic GMP might be involved in the cholinergic response of the pyramidal cells.

A variety of procedures have been used to produce seizure and epileptiform activity in the hippocampus. Similar types of interactions carried out on the hippocampus also evoke epileptiform and synchronous electrical activity in the transplants, suggesting the low seizure threshold of the hippocampus is, at least in part, due to intrinsic pathways within the hippocampus. Electrical stimulation of the transplant, application of penicillin and microiontophoretic application of cobalt could be used to elicit such hypersynchronous and epileptiform (interictal spike) activity from the hippocampal transplants. Additionally, such activity could only be recorded from the transplant itself and exhibited many of the characteristics and pharmacological sensitivities which have been reported in situ. Application of such substances as diazepam, barbituates or local anesthetics antagonized the seizures produced within the transplants. Additionally, such characteristics as phase reversal of the hypersynchronous discharge and the postictal period were easily demonstrated in the transplants.

Significance to Bio-medical Research and the Program of the Institute:

This project has yielded basis information into the requirements for neuronal development of the hippocampus as well as providing a useful tool in which to study not only the development and characteristics of epileptogenic activity but also certain of the basic mechanisms through which such seizure activity develops.

Proposed Course:

This project is terminated with the resignation of the PI.

Publications:

Lars Olson, Robert Freedman, Ake Seiger and Barry Hoffer, Electrophysiology and Cytology of Hippocampal Formation Transplants in the Anterior Chamber of Eye. I. Intrinsic Organization. Brain Research, 119: 87-106, 1977.

B. Hoffer, A. Seiger, R. Freedman, L. Olson, and D. Taylor, Electrophysiology and Cytology of Hippocampal Formation Transplants in the Anterior Chamber of the Eye. II. Cholinergic Mechanisms. Brain Research, 119: 107-132, 1977.

Barry J. Hoffer, Ake Seiger, David Taylor, Lars Olson and Robert Freedman, Seizures and Related Epileptiform Activity in Hippocampus Transplanted to the Anterior Chamber of the Eye. I. Characterization of Seizures, Interictal spikes and Synchronous activity. Experimental Neurology, 54: 233-250, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01316-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)
Ultrastructure of the synaptic connections to the tissues transplanted in oculo.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David A. Taylor Ph.D. SMRN NIMH

COOPERATING UNITS (if any)
Lars Olson and Ake Seiger, Karolinska Institute, Stockholm, Sweden.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Developmental Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Entire fetal rat hearts transplanted to the anterior chamber of the eye proliferate and mature into adult hearts, maintaining the rhythmical activity associated with cardiac muscle. Ultrastructural and cytological examination of such transplants revealed the presence of cholinesterase positive fibers as well as formaldehyde-induced fluorescent nerve fibers in the transplant. Additonally, mylinated nerve fibers coursed throughout the transplants as well as nerve fibers which contained vesicular-like material presumed to be noradrenergic nerve fibers.

Project Description:

Objective:

The objective of this project was to determine if electrophysiologically demonstrable synaptic connnections between the nerve fibers of the host iris and the transplant itself could be observed microscopically. These data would demonstrate that such in oculo transplants did receive functional synaptic input from the host iris which could be observed at the sub-cellular level.

Methods Employed:

Entire fetal rat hearts were transplanted to the anterior chamber of the eye. Following a period of approximately 1 month in oculo, such transplants were tested for the ability of physiological intervention to alter the in oculo cardiac rate. Electrical recordings were obtained through single barrel micropipettes and the rate of action potential discharge recorded before, during and after activation of the pupillo-constrictor reflex to the host iris (i.e. shining light into the eye). Several transplants were obtained for electron microscopic analysis by perfusing the animals, removing the transplants and conducting normal fixation and staining techniques of the electron microscope. Ultrathin sections were obtained by an ultramicrotome and the sections poststained in uranyl acetate, lead citrate. Such sections were then observed in a Phillips 200 electron microscope.

Results:

Physiological activation of the pupilloconstrictor reflex results in a reduction of intraocular heart rate. Since this activation leads to increased neuronal activity of the cholinergic nerve fibers to the host iris a reduction in heart rate of the transplant corresponds to the action expected for acetylcholine in this preparation. Additional studies, such as the blockade of this response by either iontophoretically applied magnesium ion and reduction of the response by removal of the ciliary ganglion suggests that this action is a synaptically mediated event.

Cytological examination of these transplants reveals the presence of presumably cholinergic as well as noradrenergic nerve fibers. In addition, the pattern of innervation for the respective nerve fibers is remarkably similar to that reported in situ, there being a dense noradrenergic and cholinergic innervation to the atrial portion of the transplant and a sparse noradrenergic and almost non-existant cholinergic innervation to the ventricular portion.

Ultrastructural examination of thin sections obtained from the transplants revealed the main characteristics of adult cardiac muscle, with intercalated discs and all the major divisions of the myofibril being present (e.g. Z-band, A-band, etc.). Thus the cardiac muscle observed in the transplants closely resembles its counterpart in the adult heart.

In addition, myelinated nerve fibers were present in various parts of the transplant as were fibers with vesicular-like inclusions suggestive of noradrenergic innervation.

Significance to Bio-medical Research and to the Program of the Institute:

This study demonstrates that tissues transplanted to the anterior chamber of the eye develop functional synaptic connections with nerve fibers derived from the autonomic ground plexus of the host iris. Additionally the presence of such nerve fibers are demonstrable even at the electron microscopic level.

Proposed Course:

This project is terminated with the resignation of the PI.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01317-01 SMRN

PERIOD COVERED
July 1, 1976 to September 30, 1977.

TITLE OF PROJECT (80 characters or less)

Nucleotides and cerebral cortical neurones.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David A. Taylor Ph.D. SMRN NIMH

COOPERATING UNITS (if any)
Trevor Stone, University of Aberdeen, Aberdeen, Scotland.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Developmental Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An identified class of neurons in the cerebral cortex respond characteristically to both acetylcholine and norepinephrine. The increase in cell firing produced by acetylcholine is closely mimicked by cyclic GMP while the depressed of cell firing produced by norepinephrine was mimicked by cyclic AMP. Other nucleotides (e.g. 5'AMP and adenosine) also produce profound depression of these same neurons which can, however, be selectively blocked by theophylline. In addition, simultaneous application of both norepinephrine and either 5'AMP or adenosine by microiontophoresis result in a synergistic action of both depressants, suggesting that these two substances (norepinephrine and 5'AMP or adenosine) have an interaction in the cerebral cortex which may be quite important in modulating the output of these neurons.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objective of this project was to investigate the role which cyclic nucleotides may have in the functioning of cells in the cerebral cortex. In particular, the possibility that cyclic AMP and cyclic GMP may be involved in mediating the opposing actions of norepinephrine and acetylcholine respectively.

Methods Employed:

Animals were anesthetized with urethane and placed in a stereotaxic frame. Neuronal activity from identified pyramidal tract neurons in the cerebral cortex was recorded using conventional electrophysiological techniques. Substances were applied to these neurones by microiontophoresis and responses to various drugs summed using a PDP-12 computer and appropiate software. In some cases, the interaction of substances with iontophoretically applied drugs was studied utilizing parenteral injection of substances into the animal.

Major Findings:

Acetylcholine produced a rapid excitation of cerebral cortical neurones similar to that which has previously been reported. Cyclic GMP excited approximately 75% of the cells studied and exhibited a remarkable correlation with respect to similar actions of acetylcholine. Microiontophoretically applied atropine abolished the response to acetylcholine without altering the cyclic GMP excitation, simultaneous administration of calcium ion failed to block the cyclic GMP response. Application of cyclic GMP by electroosmosis (a technique requiring ejection of the substance with opposite current) produced an excitation of the pyramidal tract neurones as well. Likewise, careful current controls carried out on some cells which responded to cyclic GMP revealed no effect of current. These data suggested that cyclic GMP might act as an intracellular mediator of the acetylcholine response.

Norepinephrine produced a relatively uniform, consistent depression of neuronal firing in a majority of these cells. Cyclic AMP exhibited a marked correlation with the responses of these neurons to norepinephrine. In addition, application of a phosphodiesterase inhibitor through another barrel of the micropipette markedly potentiated the response observed to norepinephrine. These data suggested that cyclic AMP might be involved in mediating the response of cerebral cortical neurons to norepinephrine.

Other adenine nucleotides (e.g. adenosine and 5'AMP) were

used to test the possibility that the action of cyclic AMP might be due to the ability of this substance to interact with an extracellular adenosine receptor. Considerable amount of biochemical evidence suggests that this receptor can also activate adenylate cyclase. By administering theophylline parenterally to the animal, however, a selective blockade of 5'AMP or adenosine was observed with the depression of cell firing by cyclic AMP still present. These data suggested that even though cyclic AMP could interact with the adenosine receptor its action could not be attributed only to this effect and led to the suggestion that cyclic AMP might be the intracellular mediator for norepinephrine in the cerebral cortex.

Proposed Course:

This project will terminate with the resignation of the PI.

Publications:

T. W. Stone and D.A. Taylor: Microiontophoretic studies of the effects of cyclic nucleotides on the excitability of neurones in the rat cerebral cortex. J. Physiol. 266: 523-543, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01481-04 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Short and long term regulation of tryptophan hydroxylase in brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Meek	Pharmacologist	SMRP NIMH
Other:	E. Marco	Visiting Fellow	SMRP NIMH
	L. Neckers	Staff Fellow	SMRC NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Techniques for studying serotonin turnover in discrete rat brain nuclei were developed in order to study short term changes in 5HT synthesis elicited by psychotropic drug. These methods involved measurement by high pressure liquid chromatography of changes in serotonin, 5-hydroxytryptophan or 5-hydroxyindoleacetic acid concentrations after pharmacologically blocking their metabolism, synthesis or efflux, respectively.

PHS-6040
(Rev. 10-76)

Project Description:

It has been known for some years that the turnover of serotonin in brain is under dynamic control, and it has been generally assumed that the changes seen in turnover reflect changes in firing rate of the serotonergic neurons because stimulation of raphic nuclei causes an accumulation of 5HIAA in brain. Therefore, in order to gain insight into factors which affect serotonergic neurons, many workers have examined effects of pharmacological and physiological manipulations on the 5HT turnover in whole rat brains, or large brain areas.

The major objectives of the current studies were to develop new techniques which would allow study of 5HT turnover in discrete brain nuclei and to apply this new methodology to (1) the study of a clinically important class of drugs, the antidepressants and (2) to gain direct information as to whether an increase in firing rate actually produces a corresponding increase in 5HT synthesis.

In order to achieve these goals, methods of great sensitivity were required to be able to measure 5HT, 5HIAA and 5HTP in discrete nuclei. High pressure liquid chromatography (HPLC) methods previously developed in this lab (Z01 MH 014181-02 SMRP) were adapted and perfected to permit these measurements to be simply carried out. The turnover of 5HT can be studied by following the metabolic transformation of labelled tryptophan into 5HT, or by one of five nonisotopic techniques which involve pharmacologically altering the synthesis or breakdown of 5HT, or 5HTP, or the transport of the 5HT metabolite, 5HIAA. We examined all 5 nonisotopic turnover methods in discrete nuclei, both those whose 5HT is contained only in nerve terminals and those rich in 5HT cell bodies. We found that the two methods of choice for studying turnover were to measure the 5HIAA accumulating after probenecid and to measure the accumulation of 5HTP after blocking its decarboxylation. Using the latter method, we discovered that the synthesis of 5HT in the raphe nuclei (which contain 5HT cell bodies) is 30 times greater than that of an area containing only 5HT terminals (the caudate nucleus). The data indicate that turnover in the cell bodies (and possibly dendrites) is quite rapid. Currently, there is no information available on the function of 5HT in dendrites, but our results strongly indicate that it might have a function because 5HT is utilized at a high rate in cell bodies.

It is known that some of the clinically useful antidepressants change 5HT turnover, although it has not been established whether this is an essential component of their mechanism of action. We are currently showing that chlorimipramine decreases 5HT turnover and we plan to compare this 5HT changes with those elicited

by other antidepressants; in preliminary experiments we have found that nomifensine, a potent antidepressant fails to decrease 5HT turnover rate in several brain areas which are affected by chlorimipramine.

The experiments carried out with chlorimipramine are aimed at revealing whether the drug has an anatomically selective action. Using a high dose (15 mg/kg) we examined 5HTP accumulation in 5 nuclei and found that the drug reduced the 5HTP accumulation in all of these nuclei. We are now examining with a dose response study whether there is any difference in the threshold of this effect. Some antidepressants, such as desmethylimipramine which do not affect whole brain 5HT turnover may be affecting it in certain nuclei by their effects on noradrenergic inputs to these areas.

In addition to studying these antidepressants, we will attack the question of the relationship between firing rate and 5HT synthesis. Our proposed experiment is to stimulate the median and dorsal raphe nuclei separately and to measure the 5HT turnover in terminal areas receiving an input from each of these nuclei.

Publications:

Meek, J. L. and Lofstrandh, S.: Tryptophan hydroxylase in discrete brain nuclei: Comparison of activity in vitro and in vivo. Europ. J. Pharmacol. 37: 377-380, 1976.

Neckers, L. M., Bertilsson, L. and Costa, E.: The action of fenfluramine and p-chloroamphetamine on serotonergic mechanisms: A comparative study in rat brain nuclei. Neurochem. Res. 1: 29-35, 1976.

Neckers, L. M. and Meek, J. L.: Measurement of turnover rate in discrete nuclei of rat brain. Life Sci. 19: 1579-1584, 1976.

Neckers, L. M., Biggio, G., Mola, E. and Meek, J. L.: Modulation of brain tryptophan hydroxylase activity in brain tryptophan content. J. Pharmacol. Exp. Ther. 201: 110-116, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01486-03 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Release of Ca^{2+} binding phosphodiesterase activator from storage in synaptic membranes by putative neurotransmitters.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Revuelta	Visiting Associate	SMRP NIMH
Other:	P. Uzunov	Visiting Scientist	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project we studied the release of a Ca^{2+} binding endogenous protein which activates phosphodiesterase and adenylate cyclase from rat brain membrane fraction by some putative neurotransmitters. Those neurotransmitters that activate adenylate cyclase released significant quantities of activator. There was a correlation between the activity of those agents as adenylate cyclase stimulators and the degree of activator release. Serotonin, which did not stimulate adenylate cyclase activity failed to show any activator release. Dopamine was most active in both adenylate cyclase activation and activator release using preparation from caudate nucleus. Norepinephrine was much more active when membrane fraction from cerebellum was used.

The release of activator by the neurotransmitter was further used as a model to study the effect of some psychoactive drugs on this process. It was established that correlation exists between the clinical activity by those drugs and their potency to block activator release.

Project Description:

Recently we found that the endogenous protein activator of phosphodiesterase (PDEA), which plays an important role in regulation of cAMP metabolism, is membrane-stored and that it could be released by the addition of cAMP, ATP and cAMP-dependent protein kinase in the incubation mixture. This project aimed to ascertain whether some neurotransmitters that are known to activate brain adenylate cyclase could substitute for cAMP in PDEA release. The positive answer of these questions would further substantiate the role of the endogenously formed cAMP in brain as a trigger of PDEA release and as an important mechanism of self-regulation.

Norepinephrine, dopamine, histamine, serotonin and adenosine were used as potentially active substances that might produce endogenous cAMP and carry on the membrane phosphorylation and PDEA release. In order to evaluate the relative potency of the neurotransmitter studied and specificity of their effect, membrane fractions from both rat cerebellum and caudate nucleus were used. The activator release was studied using the previously developed procedure which consists of preparing the membrane fractions, incubation of this preparation in the presence of ATP and cAMP-dependent protein kinase, and isolation and measurement of free activator.

It was found that norepinephrine, dopamine, histamine and adenosine released a significant amount of PDEA from preparations isolated from whole rat brain, cerebellum or caudate nucleus. The presence of ATP in the incubating mixture was essential to cause the release of the activator. Moreover, norepinephrine showed significantly higher PDEA releasing activity in membranes prepared from cerebellum, whereas dopamine was more active in caudate nucleus. We proposed the idea that the neurotransmitters studied release PDEA by stimulation of adenylate cyclase activity and increase of cAMP, rather than by a direct effect on the activator binding sites. This was substantiated by the fact that a good correlation was found to exist between the potency of those agents as adenylate cyclase activators and their ability to promote PDEA release. For instance, serotonin failed to stimulate adenylate cyclase and to release PDEA. Dopamine was more potent as stimulant of adenylate cyclase and PDEA release in caudate nucleus. Using membranes from rat cerebellum, these neurotransmitters showed potency in reverse order.

This study supports the idea that the in vitro PDEA release could occur not only in the presence of exogenous cAMP but also under different conditions, when the concentration of the endogenous cAMP increases.

Furthermore, the in vitro PDEA release by neurotransmitters offers a relatively simple system to study the effect of drugs on this process. The development of this program in these directions gave us an opportunity to see that the pharmacologically active phenothiazine tranquilizers like chlorpromazine, trifluorperazine, clozapine, as well as the butyrophenone haloperidol, blocked the release of PDEA induced by either norepinephrine or dopamine. The pharmacologically nonactive sulfoxide analogues of trifluoperazine and chlorpromazine failed to change both the activation of adenylate cyclase and the consecutive PDEA release.

This project has been terminated.

Publications:

Revuelta, A. V., Uzunov, P. and Costa, E.: Release of phosphodiesterase activator from particulate fractions of cerebellum and striatum by putative neurotransmitters. J. Neurochem. Research 1: 217-227, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01487-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Regulation of phosphodiesterase by a protein activator

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. E. Gnegy	Staff Fellow	SMRP NIMH
Other:	P. Uzunov	Visiting Scientist	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work extends the study of the mechanism of phosphodiesterase regulation by an endogenous protein activator. The activator stored in the cell membrane fraction can be released into the cytosol by a cAMP-dependent phosphorylation of a membrane binding site.

A subcellular distribution of the localization of activator in rat brain and its release has been performed. The activator is located mainly in the mitochondrial fraction and particularly in the mitochondrial subfractions containing synaptic membranes and synaptic vesicles. Nearly 100% of the activator in these fractions can be released by the cAMP-dependent phosphorylation. The mechanism for cAMP during periods of sustained synaptic transmission and could explain post-synaptic receptor supersensitivity to neurotransmitters.

PHS-6040
(Rev. 10-76)

Project Description:

In previous studies we have shown that an endogenous protein activator located in the cell membranes can be released into the cytosol by a cAMP-dependent phosphorylation of a membrane binding site. We demonstrated that the protein phosphorylated is not the activator itself but is most likely its binding site in the membrane. Furthermore, the release of the activator did not involve a cGMP-dependent phosphorylation.

We performed a complete subcellular distribution of the activator localization in rat brain and also its release from the membrane fractions. The activator was located largely in the mitochondrial and microsomal fractions with very little being in the nuclear fraction. Only the activator in the mitochondrial fraction could be readily released by the cAMP-dependent phosphorylation mechanism. Further purification of the mitochondrial fraction showed that the activator was concentrated in fractions containing synaptic membranes and another containing synaptic vesicles and membranes. Nearly 100% of the activator in these fractions can be released. There was no activator located in the pellet mitochondria.

The activator in brain is mainly stored in membranes involved in the process of synaptic transmission. It appears that the activator stores are readily releasable through membrane phosphorylation. Though the activator was also concentrated in the microsomal fraction, it could not be released from this fraction. It is possible, therefore, that the release of activator changes the Vm of phosphodiesterase thereby regulates the cAMP concentration during periods of sustained synaptic transmission when there is a large elevation in cAMP. This phenomenon could also help explain the molecular mechanism of sub- or supersensitivity to neurotransmitters. An alteration in the release of activator in the membranes could affect both the adenylate cyclase activity in the membrane and increase or decrease the phosphodiesterase activity in the cytosol. We are now studying whether drug tolerance involves a mechanism of activator release.

Publications:

Uzunov, P., Gnegy, M., Lehne, R., Revuelta, A. and Costa, E.: A neurobiological role for a protein activator of cyclic nucleotide phosphodiesterase. In Costa, E., Giacobini, E. and Paoletti R. (Eds.): Advances in Biochemical Psychopharmacology. Vol. 15, New York, Raven Press, 1976, pp. 283-301.

Gnegy, M. E., Nathanson, J. A. and Uzunov, P.: Release of the phosphodiesterase activator by cyclic AMP-dependent ATP: Protein phosphodiesterase from subcellular fractions of rat brain. Biochim. Biophys. Acta 497: 75-85, 1977.

Costa, E., Gnegy, M. E. and Uzunov, P.: Regulation of dopamine receptor sensitivity by an endogenous protein activator or adenylate cyclase. Naunyn-Schmied. Arch. Pharmacol. 297: S47-S48, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01489-04 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanisms of trans-synaptic control of protein synthesis in postsynaptic neurons.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Chuang	Staff Fellow	SMRP NIMH
Other:	R. A. Hollenbeck	Guest Worker	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have further explored the molecular mechanisms that induce tyrosine hydroxylase (tyrosine-3-monooxygenase) transsynaptically in rat adrenal medulla. The present investigation has emphasized the detailed mechanisms of the uptake by nuclei of the catalytic subunits of cytosol ATP: protein phosphotransferase (protein kinase, PK). We have provided evidence that the catalytic subunits of cytosol PK can be taken up into isolated nuclei following dissociation of the holoenzyme by cAMP in vitro. These newly taken up catalytic subunits of PK are tightly bound to chromatin and phosphorylate primarily the nonhistone proteins of chromatin. RNA synthesis in isolated nuclei was significantly enhanced following the uptake of PK and enhanced phosphorylation of nuclear proteins. The PK's endogenously present in medullary nuclei were also isolated and characterized.

PHS-6040
(Rev. 10-76)

Project Description:

Induction of tyrosine hydroxylase in rat adrenal medulla is an ideal system for the study of gene regulation exerted by neurotransmitters impinging upon receptors on post-synaptic cells. Earlier studies in this laboratory have obtained solid evidence which suggests that increased release of acetylcholine from the cholinergic axon elicited by stimulus triggers a sequence of biological events which ultimately enhance the rate of tyrosine hydroxylase biosynthesis in the chromaffin cells. These cascade mechanisms involve an uptake by the nuclei of the catalytic subunits of cyclic adenosine 3',5' monophosphate (cAMP) dependent protein kinase (PK) in the cytosol, resulting in an increased synthesis of heterogenous poly A containing RNA in nuclei. The present study was addressed to establish an in vitro system to investigate the uptake of cytosol cAMP dependent PK into isolated nuclei in an attempt to understand the molecular mechanisms of the PK translocation and to obtain further support for the physiological importance of the transfer of cytosol PK to nuclei.

We have found that when nuclei purified from beef adrenal medulla were incubated with cAMP and cAMP dependent PK isolated from beef heart or beef adrenal medulla, the phosphorylation of nuclear proteins was enhanced by more than 100%. The main substrates for the endogenous and newly taken up PK were nonhistone proteins which were bound to chromatin. The activity of PK which was tightly bound to chromatin was markedly enhanced following the incubation of nuclei with cAMP and cAMP dependent PK. This enhanced nuclear PK activity was cAMP independent and preferred histone to casein as the phosphate acceptor. Moreover, the enhanced PK activity was nullified by the addition of purified regulatory subunits derived from cytosol cAMP dependent PK and this inhibition was reversed by the addition of cAMP. Thus it appears that the catalytic subunits of cytosol PK were taken up into nuclei following the dissociation of the holoenzyme by cAMP in the incubation medium. The rate of this PK uptake was temperature dependent and was 3 times faster than that of other labelled protein from medullary cytosol. We found that the capacity of the isolated nuclei to synthesize RNA was significantly enhanced when more than 3 pmole of phosphate was incorporated into one microgram of nuclear protein by the translocated catalytic subunits of cytosol PK. We have previously demonstrated that phosphorylation of the non-histone protein of beef adrenal medulla chromatin enhanced the template activity of this chromatin. Such increase of RNA synthesis was catalyzed by homologous RNA polymerase II - the enyzme responsible for the synthesis of messenger RNA in vivo. Thus, our present results are compatible with the view that translocation of catalytic subunits of PK enhanced RNA synthesis in isolated nuclei by a re-

lease of template restriction through phosphorylation of chromosomal proteins.

In order to better understand the control of gene expression by nuclear phosphorylation, we have characterized the endogenous protein kinases present in nuclei isolated from beef adrenal medulla. Three forms of PKs were isolated from medullary nuclei using ion exchange column chromatography. These kinases were cAMP independent and appeared to use either casein or histone as the preferential phosphate acceptor. However, they differed from the catalytic subunits of cytosol PK in their substrate specificities, sedimentation constants and column elution profiles. Moreover, the activities of these multiple forms of nuclear PK failed to be nullified by the regulatory subunits of cytosol PK and were resistant to the endogenous inhibitor of cAMP dependent PK. Thus it appears impossible to explain a cAMP mediated phosphorylation of nuclear protein in vivo through an action of cAMP on nuclear PK. However, it seems logical that the nuclear uptake of dissociated catalytic subunits would be an ideal way whereby stimuli impinging on specific cell membrane receptors can regulate gene activity.

To further explore the detailed mechanisms of tyrosine hydroxylase induction mediated by an increased level of cAMP, we are extending our study using cultured neuroblastoma cells in which cAMP content is increased by the addition of a phosphodiesterase inhibitor (RO-20-1724) chemically related to theophylline. We are attempting to elucidate whether the tyrosine hydroxylase induction also in the neuroblastoma cells involves an uptake of cytosol PK catalytic subunits and subsequent phosphorylation of specific nuclear proteins. Our ultimate goal would be that of illustrating, that the messenger RNA specific to tyrosine hydroxylase is indeed accumulated during the induction of the protein molecule.

Publications:

Chuang, D. M. and Costa, E.: Trans-synaptic regulation of RNA synthesis in adrenal medulla of rats exposed to cold. In Costa, E., Giacobini, E. and Paoletti, R. (Eds.): Advances in Biochemical Psychopharmacology. Vol. 15, New York, Raven Press, 1976, pp. 429-444.

Chuang, D. M. and Costa, E.: Trans-synaptic regulation of ribonucleic acid biosynthesis in rat adrenal medulla. Mol. Pharmacol. 12: 514-518, 1976.

Chuang, D. M., Hollenbeck, R. A. and Costa, E.: Enhancement of template activity in chromatin from adrenal medulla following phosphorylation of chromatin proteins. Science 193: 60-62, 1976.

Costa, E., Chuang, D. M., Guidotti, A. and Hollenbeck, R.: Control of nuclear function in chromaffin cells by persistent activation of nicotinic receptors. In Jenden, D. J. (Ed.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01490-03 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Deuterium-choline: Evaluation of possible isotope effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. K. Garrison	Staff Fellow	SMRP, NIMH
Other:	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP, NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Choline containing deuterium in various positions is often used as a marker to study the rate of formation and metabolism of acetylcholine in vivo. Our objective was to evaluate in vitro the possible isotope effect of various deuterium-choline isotopes on choline acetyltransferase and cholinesterase.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

Publications:

Garrison-Gund, C. K. and Neff, N. H.: Deuterium-labeled choline: Lack of isotope effects during the synthesis and catabolism of acetylcholine in vivo. Neurochem. Res. 1: 679-682, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01491-03 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Rat Pineal Cyclic Adenosine 3',5'-Monophosphate Phosphodiesterase Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Oleshansky	Commissioned Officer	SMRP,NIMH
Other:	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP,NIMH

COOPERATING UNITS (if any)
None

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NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.6	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Repeated stimulation of the beta-adrenergic receptors of the rat pineal gland results in a loss of responsiveness of the cyclic adenosine 3',5'-monophosphate (cAMP) system of the gland. We determined if less cAMP accumulated in rat pineal in vivo after a second dose of l-isoproterenol, and whether the changes observed could be explained as the consequences of decreased adenylate cyclase activity and/or increased low Km phosphodiesterase activity.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01492-05 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Properties of Monoamine Oxidases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP,NIMH
Other:	R. J. Wyatt	Chief, Laboratory of Clinical Psychopharmacology	SMRC,NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

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Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the biochemical basis for the various monoamine oxidases (E.C. 1.4.3.4.). Our present interests are: (1) To identify the changes that occur in monoamine oxidase activity with age; (2) to determine the characteristics of cardiovascular amine oxidase and compare them with the enzymes in neuronal tissues and (3) to continue our survey of the types and activities of monoamine oxidase in autopsy material from individuals with various psychiatric disorders.

PHS-6040
(Rev. 10-76)

Project Description:

The monoamine oxidases (E.C. 1.4.3.4.:MAO) are enzymes which participate in the inactivation of biogenic amines. Drugs that inhibit MAO are used clinically as antidepressants and antihypertensives. The identification of type A and B MAO (see M-SMR-P-12, 72-72) has prompted studies to define the role of these enzymes. Our present objectives were: 1) To identify the changes that occur in monoamine oxidase activity with age; (2) to determine the characteristics of cardiovascular amine oxidases and compare them with the enzymes in neuronal tissues and (3) to continue our survey of the types and activities of MAO in autopsy material from individuals with various psychiatric disorders.

MAO activity was determined using several amine substrates. Type A activity was identified by using the specific substrate norepinephrine or serotonin while type B enzyme was identified with phenylethylamine (PEA).

We found that the heart of aged rats (24 months old) showed increased activity of both type A and B MAO. Serotonin deamination increased six-fold and PEA deamination eleven-fold over values for young rats (6 weeks old). In contrast to heart, the deamination of both amines was decreased in the vessels of the aged rats. Serotonin deamination declined by over twelve-fold in mesenteric artery and six-fold in aorta. PEA deamination in vessels was decreased to 36 and 55 percent of the values found for young rats for mesenteric artery and aorta, respectively. Type A MAO is associated almost exclusively with sympathetic neurons in blood vessels. The decline of type A activity in the vessels of aged animals may reflect a decrease in the density of sympathetic neurons innervating the vasculature, or perhaps an age-related alteration in the metabolic properties of sympathetic nerve endings.

The cardiovascular tissues appear to contain amine oxidases that are quite different from those of brain. Pargyline, a MAO inhibitor, when tested in vitro or administered to rats, did not differentially block the deamination of norepinephrine and PEA as it does in brain. Moreover, there is a PEA oxidase activity in periphery that is blocked by pargyline and an activity that is resistant to pargyline but blocked by semi-carbazide or ci[illegible]ri zone. PEA oxidase activity can be differentiated from norepi[illegible]e-phrine oxidase activity by thermal stability. The pargyline-resistant PEA oxidase may be related to a connective tissue enzyme. Studies of the types of enzymes associated with the vasculature is of interest because of the evidence that low MAO activity is found in human platelets from schizophrenics and the low activity may have a heritable component.

Our study of human tissues for MAO activity in autopsy material is not yet complete.

MAO inhibitor drugs are used to treat hypertension and depression. Depression and hypertension are often associated with aging. Therefore, it is important to characterize the enzymes of the cardiovascular system and to determine if they change with age. Our studies may lead to a better understanding of depression and hypertension and how drugs such as MAO inhibitor might alleviate these disorders.

We will continue our investigation of the biochemical basis for type A and type A MAO and their possible role in neuronal function.

Publications:

Neff, N. H. and Fuentes, J. A.: The use of selective monoamine oxidase inhibitor drugs for evaluating pharmacological and physiological mechanisms. Ciba Symposium 39: 163-172, 1972.

Neff, N. H., Garrison, C. K., Seaborg, J. B., Fuentes, J. and Wyatt, R. J.: Trace amines and the monoamine oxidases. In Usdin, E. and Sandler, M. (Eds.): Trace Amines and the Brain. New York, Marcel Dekkar, Inc., 1976, pp. 41-57.

Fuentes, J. A. and Neff, N. H.: Inhibition by pargyline of cardiovascular amine oxidase activity. Biochem. Pharmacol. In press.

Trepel, J. B., Wyatt, R. J. and Neff, N. H.: Differential distribution of type A and B monoamine oxidase in neuronal tissues. Eur. J. Pharmacol. 43: 373-375, 1977.

Fuentes, J. A., Trepel, J. B. and Neff, N. H.: Monoamine oxidase activity in the cardiovascular system of young and aged rats. Exp. Gerontol., In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01497-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Using Stable Isotopes and Mass Fragmentography to Study Monoamine Biosynthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Galli	Visiting Associate	SMRP NIMH
Other:	J. W. Commissiong	Visiting Fellow	SMRP NIMH
	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to utilize the sensitivity and specificity of gas chromatography-mass spectrometry to study problems related to mental health. Our present investigations entail administering $^{18}O_2$ to rats and following its incorporation into tyrosine and then into dopamine and its metabolites.

Project Description:

Our objective was to administer the stable isotope $^{18}O_2$ to rats so it could be incorporated into body constituents and the rate of its incorporation could be followed with time. The kinetics of the appearance and loss of ^{18}O-containing endogenous materials will allow us to evaluate the dynamic state of specific biochemical pathways. Our present studies deal with the incorporation of $^{18}O_2$ into tyrosine and into dopamine and its metabolites.

Oxygen-18 is produced by the electrolysis of water containing-^{18}O (95 atom percent). The $^{18}O_2$ produced is administered to unrestrained rats in a closed homeostatic chamber. Oxygen-18-containing endogenous materials are assayed by gas chromatography-mass spectrometry (GC-MS).

Our initial studies concerned the potential use of $^{18}O_2$ incorporation into dopamine as a procedure for studying dopamine metabolism in man. Pilot studies revealed that the decline of ^{18}O-dopamine from rat brain after exposure to $^{18}O_2$ was different from our previous results using radioactive or pharmacological techniques. The calculated rates were slower than anticipated. A slower decline of ^{18}O-dopamine could have been the consequence of ^{18}O-tyrosine being converted to ^{18}O-DOPA and then to ^{18}O-dopamine during after the animals were removed from the $^{18}O_2$ atmosphere. The ^{18}O atom in this situation would be on the 4th position and ^{16}O on the 3rd position of the aromatic ring. It is important to call to mind that the GC-MS procedures that are currently used to study catechol compounds cannot identify the position of the ^{18}O atom on the aromatic ring.

Dopamine containing two atoms of ^{18}O is found in rat brain after 60 min of exposure to $^{18}O_2$. This could only occur if ^{18}O was first incorporated into phenylalanine by phenylalanine hydroxylase and then a second atom of ^{18}O added when tyrosine was converted to DOPA by tyrosine hydroxylase. 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) containing a single atom of ^{18}O were also present in rat brain, but as already mentioned, the GC-MS cannot distinguish whether ^{18}O is at position 3 or 4 of the aromatic ring. The basic problem which restricts the usefulness of this technique for estimating catecholamine turnover in animals and in man is in interpreting the meaning of the change of ^{18}O-dopamine and its metabolite after exposure to $^{18}O_2$. Currently, it is our opinion that although the $^{18}O_2$ procedure originally appeared promising for studying the formation of catecholamines in man, our most recent studies in animals suggest that it should not be considered as a method of choice for evaluating catecholamine formation in man at the present time.

In the course of our studies of catecholamines, it was necessary to develop a sensitive GC-MS procedure for the assay of phenylalanine and tyrosine in tissues. These experiments have led to a promising procedure for identifying heterozygous phenylketonurics. Substantial quantities of ^{18}O-tyrosine are formed in vivo during one hour of exposure to $^{18}O_2$. To test whether the incorporation of $^{18}O_2$ was a result of the enzymatic hydroxylation of phenylalanine by phenylalanine hydroxylase, rats were treated with the phenylalanine hydroxylase inhibitor, p-chlorophenylalanine, 24 hours prior to exposure to $^{18}O_2$. As a result of this treatment, there was a significant fall of the levels of ^{18}O-tyrosine in plasma and striatum. Thus, there is the potential for evaluating the activity of phenylalanine hydroxylase in vivo in human subjects from samples of plasma after breathing $^{18}O_2$. Homozygotes for phenylketonuria are readily detected, but heterozygotes, because of alternated enzyme activity, cannot usually be identified by simple means. The $^{18}O_2$ procedure could be performed under steady-state conditions and, thus, eliminate the need to use radioactive phenylalanine or administer large amounts of phenylalanine, which upsets the normal balance of amino acids in the body, to follow the formation of tyrosine as it has been done in the past to identify heteroxygotes for phenylketonuria.

Abnormal metabolism of the biogenic amines has been implicated in several psychiatric disorders. As a laboratory of preclinical pharmacology, we are attempting to provide the means whereby the biological psychiatrist can evaluate amine metabolism in man with minimal discomfort and danger to the patient.

Our future studies will be concerned with the potential use of $^{18}O_2$ for studying amine metabolism in nuclei of brain.

Publications:

Neff, N. H., Galli, C. L. and Costa, E.: The turnover of dopamine: Studies with $^{18}O_2$. In Costa, E. and Giacobini, E. (Eds): Advances in Biochemical Psychopharmacology. Vol. 16, New York, Raven Press, 1977, pp. 187-192.

Galli, C. L., Commissiong, J. W. and Neff, N. H.: Measuring the formation of the biogenic amines utilizing $^{18}O_2$. Biochem. Pharmacol., in press, 1977.

Neff, N. H., Commissiong, J. W., Galli, C. L., Ivuone, P. M., Garrison-Gund, C. and Karoum, F.: Mass spectrometry to measure regional aspects of dopamine metabolism. In Usdin, E. and Weiner, N. (Eds.): Biochemistry and Function of Monoamine Enzymes. New York, Marcel-Dekker Inc., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01498-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Post tetanic potentiation and assessment of anxiolytic and anticonvulsant drug actions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Suria	Staff Fellow	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Pre-clinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Diazepam inhibits post tetanic potentiation of fast excitatory post synaptic potentials in bullfrog sympathetic ganglia, without altering the responses to single shocks. In addition, diazepam, like GABA and dibutyryl GMP depolarizes presynaptic nerve terminals, that can be antagonized by picrotoxin and not by tubocurarine or atropine. The bullfrog and rat superior cervical ganglion (SCG) accumulate exogenous radiolabelled GABA presumably in the glial cells. This radioactive GABA can be released by dibutyryl cGMP, high concentration of K^+, and diazepam. Rat SCG contains endogenous GABA that can be altered by various drugs and other treatments, such as amino-oxyacetic acid, urethane, decentralization and electrical stimulation. We postulate that diazepam acts presumably by releasing glial GABA which depolarizes presynaptic terminals and inhibits transmitter release, thus inhibiting post tetanic potentiation.

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01499-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Action of benzodiazepine on the second messengers of bullfrog sympathetic ganglion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard Lehne	Laboratory Technician	SMRP NIMH
Other:	Amin Suria	Staff Fellow	SMRP NIMH
	Petko Uzunov	Visiting Scientist	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

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NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES [X] (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this work we explored the mechanism of cAMP increase in Bullfrog sympathetic ganglia by a group of benzodiazepine analogues. Briefly, the positive findings are: 1) In Bullfrog sympathetic ganglia there are multiple forms of phosphodiesterase (PDE); 2) The pharmacologically active drugs inhibit PDE and do not change adenylate cyclase activity. The degree of inhibition is higher when PDE is activated by the endogenous protein activator. Most benzodiazepines have Ki value 10 to 40 times below the Km of PDE using cAMP as a substrate, whereas the Ki values are 2 to 60 times higher than the Km of PDE when cyclic 3',5' guanosine monophosphate (cGMP) is a substrate. This might explain the selective increase of cAMP but not cGMP by benzodiazepines.

The increase of ganglionic cAMP by the benzodiazepines might be at least in part the mechanism by which these drugs modify synaptic transmission.

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01500-05 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mass fragmentographic studies of indolealkylamines in the central nervous system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Galli	Visiting Associate	SMRP NIMH
Other:	J. L. Commissiong	Visiting Fellow	SMRP NIMH
	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective is to identify and quantitate known and novel indolealkylamines in neuronal tissue. Presently we have administered $^{18}O_2$ to rats and followed its incorporation into serotonin.

PHS-6040
(Rev. 10-76)

Project Description:

Molecular oxygen is incorporated into the 5th position of the indole ring of serotonin, thus it should be possible to study the incorporation of $^{18}O_2$ into serotonin by using gas chromatography-mass spectrometry (GC-MS) methods.

Oxygen-18 was produced by the electrolysis of water containing ^{18}O (95 atom percent). The $^{18}O_2$ produced was administered to unrestrained rats in a closed homeostatic chamber. Endogenous serotonin containing oxygen-18 was analyzed by GC-MS.

After 60 minutes of exposure to $^{18}O_2$ rat striatum contained about 22 percent of serotonin with incorporated oxygen-18. Our initial study demonstrated that molecular oxygen is indeed the source of oxygen in serotonin and that it is possible to label as well as study the metabolism of endogenous serotonin with the stable isotope, oxygen-18.

Abnormal serotonin metabolism has been implicated in several psychiatric and sleep disorders. Presently there is no reliable method for studying serotonin metabolism in the brain of man. The possibility exists that by administering $^{18}O_2$ to man and analyzing the oxygen-18 incorporated into serotonin found in spinal fluid and plasma will be a useful method of evaluating serotonin formation in man.

In the future a detailed analysis of ^{18}O-incorporation into rat brain serotonin will be attempted. In addition, drugs that are known to alter serotonin metabolism will be evaluated in our test system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01502-05 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Mass fragmentographic studies of catecholamines in discrete brain nuclei.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Koslow	Pharmacologist	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)
SMRN-IR-MH, Department of Neurology and Pediatrics, John Hopkins Hospital, Lab. Neurochemistry, NIMH, Bethesda, Maryland 20014

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective was to study the metabolism of the catecholamines and indolealkylamines in a patient with a dihydropteridine reductase deficiency. We found homovanillic acid levels low in CSF and in a brain biopsy low levels of dopamine and normal levels of norepinephrine.

This project has been terminated.

Publications:

Koslow, S. H.: Mass fragmentographic analysis of SIF cell catecholamines of normal and experimental rat sympathetic ganglia. In Eranko, O. (Ed.): SIF Cells--Structure and Function of the Small, Intensely Fluorescent Sympathetic Cells. Fogarty International Center Proceedings No. 30, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01503-03 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacological studies of acetylcholine turnover: Antipsychotics and antidepressants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Robinson	Staff Fellow	SMRP NIMH
Other:	D. L. Cheney	Chief, Section on Molecular Pharmacodynamics	SMRP NIMH
	F. Moroni	Visiting Associate	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cataleptogenic antipsychotic drugs, haloperidol and chlorpromazine increased the turnover rate of acetylcholine in the nucleus caudatus and nucleus accumbens whereas the noncataleptogenic antipsychotic drug clozapine had no effect on the turnover rate of acetylcholine. Both types of antipsychotic drugs decreased the turnover rate of acetylcholine in the globus pallidus. Tolerance to the effects in the three nuclei developed with chronic administration of each of the three drugs.

The antidepressant drugs had no consistent effect on acetylcholine turnover. Desipramine and chlorimipramine decreased turnover in the striatum and hippocampus. Tolerance developed to the effects of desipramine after chronic treatment. Iprindole increased the turnover rate in the hippocampus. Nomifensine increased the turnover rate of acetylcholine in the cortex, hippocampus and diencephalon, as did amphetamine. Amitriptyline had no effect on the turnover rate of acetylcholine in the areas studied.

Project Description:

Our objective was to study the effect of centrally-active drugs on the turnover rate of acetylcholine in specific brain areas.

Method of determining the concentration and turnover rate of acetylcholine were described in the Project Z01 MH 01509-08 SMRP, 1976.

Acute administration of haloperidol (10 µmoles/kg; 60 min; i.p.) and chlorpromazine (20 µmoles/kg; 60 min; i.p.), antipsychotic drugs known to cause extrapyramidal disturbances, resulted in an increase in the turnover rate of acetylcholine in the nucleus caudatus and nucleus accumbens and a decrease in the turnover rate of acetylcholine in the globus pallidus. On the other hand, clozapine (30 µmol/kg; 60 min; i.p.) which did not cause extrapyramidal disturbances, had no effect on the turnover rate of acetylcholine in the nucleus caudatus and nucleus accumbens although it decreased the levels of acetylcholine when given in large amounts. Clozapine, like haloperidol and chlorpromazine decreased the turnover rate of acetylcholine in the globus pallidus. After seven days' treatment, the turnover rate of acetylcholine showed tolerance to the facilitory action of haloperidol and chlorpromazine in the nucleus caudatus and nucleus accumbens and to the inhibitory action of all three antipsychotic drugs. It appeared that the increase in turnover rate of acetylcholine in the nucleus caudatus and nucleus accumbens was related to the extrapyramidal disturbances elicited by certain antipsychotic drugs. The effects on acetylcholine turnover rate in the nucleus caudatus, nucleus accumbens, and globus pallidus did not seem related to therapeutic activity of the drugs since tolerance developed with chronic treatment.

The acute administration of antidepressant drugs did not elicit any consistent effect on the levels and turnover rate of acetylcholine. Desipramine (33 µmoles/kg; 60 min; i.p.) and chlorimipramine (57 µmoles; 30 min; i.p.) decreased the turnover rate of acetylcholine in the striatum and hippocampus. That this effect was not related to the therapeutic action of these drugs was shown by the fact that the turnover rate of acetylcholine in the striatum and hippocampus returned to control values after treatment with desipramine (33 µmoles/kg, twice daily) for 14 or 28 days. Amitriptyline (64 µmoles/kg; 60 min; i.p.) decreased the level of acetylcholine in the cortex and the striatum but had no effect on the turnover rate of acetylcholine. These actions were similar to other drugs with anticholinergic properties Iprindole (63 µmoles/kg; 60 min; i.p.) increased the turnover rate of acetylcholine in the hippocampus. Nomifensine (71 µmoles/kg; 45 min; i.p.) increased the turnover rate of acetylcholine

in the cortex, hippocampus and diencephalon. This action is similar to the effect of amphetamine (27 μmoles/kg; 60 min; i.p.), an indirectly-acting catecholaminergic agonist, but not to apomorphine (4.8 μmoles/kg; 27 min; i.p.), a directly-acting dopaminergic agonist which had no effect on the turnover rate of acetylcholine in the cortex, hippocampus or diencephalon. Phenoxybenzamine (15 μmoles; 90 min), injected locally into the septum, blocked the nomifensine- or amphetamine-induced increase in turnover rate of acetylcholine in the cortex and hippcampus. These results suggested that the changes in turnover rate of acetylcholine in various brain areas after the administration of antidepressant drugs were a result of the effects of these agents on other neurotransmitter systems that interact with the cholinergic system, rather than a direct effect on the cholinergic system.

It has been proposed that alterations in the dynamics that regulate transmitter function at the synaptic level may be involved in various forms of mental disease. Since neuronal activity is regulated by the rates of release and inactivation of transmitters including acetylcholine, the study of the turnover rate of acetylcholine is a tool to study the functional involvement of the cholinergic system. Drugs that change cholinergic function in various brain areas of normal animals are used in the therapy of mental diseases. The in vivo study of the turnover rate of acetylcholine may yield understanding of the therapeutic effects, as well as non-therapeutic side effects, of various drugs used to treat mental disease.

Use will continue to be made of techniques to inject specific receptor inhibitors directly into specific nuclei of the rat brain and to examine the alterations of cholinergic dynamics.

Publications:

Cheney, D. L., Racagni, G. and Costa, E.: Distribution of acetylcholine and choline acetyltransferase in specific nuclei and tracts of rat brain. In Goldberg, A. and Hanin, I.(Eds.): The Biology of Cholinergic Function. New York, Raven Press, 1976.

Racagni, G., Cheney, D. L., Zsilla, G. and Costa, E.: The measurement of acetylcholine turnover rate in brain structures. Neuropharmacology 15: 723-736, 1976.

Cheney, D. L., Zsilla, G. and Costa, E.: Acetylcholine turnover rate in n. accumbens, n. caudatus, globus pallidus and substantia nigra: Action of cataleptogenic and noncataleptogenic antipsychotics. In Costa, E. and Gessa, G. L. (Eds.): Advances in Biochemical Psychopharmacology. Vol. 16, New York, Raven Press, 1977, pp. 179-186.

Cheney, D. L. and Costa, E.: Biochemical pharmacology of cholinergic neurons. Psychopharmacology - A Generation of Progress. New York, Raven Press, 1977, in press.

Costa, E. and Cheney, D. L.: Regulation of cholinergic neuron by dopaminergic terminals: Influence of cataleptogenic and non-cataleptogenic antipsychotics. Interactions Among Putative Neurotransmitters in the Brain. New York, Raven Press, 1977, in press.

Cheney. D. L. and Costa, E.: Effects of cholinergic and monoaminergic drugs on levels of and turnover of acetylcholine in the rat brain. In Butcher, L. L. (Ed.): Cholinergic - Monoaminergic Interactions in the Brain. New York, Academic Press, 1977, in press.

Robinson, S., Cheney, D. L., Moroni, F. and Costa, E.: Acetylcholine turnover in brain nuclei of rats injected with various antidepressants. In Proc. of a symposium on "Depressive Disorders", held in Rome, Italy, May 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01504-03 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cholinergic interactions: Ontogenetic and aging studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Meek	Pharmacologist	SMRP NIMH
Other:	D. L. Cheney	Chief, Section on Molecular Pharmacodynamics	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology and Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The activity of choline acetyltransferase and tryptophan hydroxylase and the concentrations of acetylcholine and serotonin in discrete nuclei of the rat brain were measured in young adult and aged rats. There was a decrease in serotonin and tryptophan hydroxylase with age in areas containing cell bodies as well as terminals. There were no changes in acetylcholine or choline content in the areas studied but there was a decrease in choline acetyltransferase activity in N. caudatus. The results suggest that neuronal destruction as a consequence of aging is selective and that the serotonergic system is more vulnerable than is the cholinergic system.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the interactions among various neuronal systems in aged rats (2 years old). Male, Sprague-Dawley rats were obtained from Zivic Miller Lab., Pittsburgh. The 24 old control rats were 24 months old and weighed 750-850 gm. The 24 young adult control rats were 35-45 days old. Both groups of rats were kept at Zivic Miller Laboratories on a diet of Purina Chow in rooms at a temperature of 18-20 C in groups of 2-4 (aged); or 8-12 (young rats) until 2-3 weeks before sacrifice. In this colony, approximately 90% of the rats survived 1 year, 25-35% for 2 years, and less than 1% for 2 1/2 years.

For measurements of ACh and Ch, rats were killed by microwave irradiation focused on the head. For other measurements, the rats were killed by decapitation. The brains were removed, frozen, and then 400 μ sections were made for removal of the nuclei as "punches". Each experiment comprised of both aged and control rats (6 per group). In one experiment, Ch and ACh were determined at the same time. In a separate experiment, 5HT was determined; tryptophan hydroxylase and CAT were measured on separate groups of rats.

5HT, ACh and Ch were determined by mass fragmentography. Tryptophan hydroxylase was measured using high pressure liquid chromatography and CAT with radiometric methods.

There was a clear loss of both 5HT and tryptophan hydroxylase in areas containing cell bodies as well as terminals. The decreased concentration of both 5HT and its rate limiting biosynthetic enzyme suggests that cell loss has occurred. The extent of the decrease was strikingly similar in all areas studied, B7, B8, B9, hippocampus and septum.

No changes could be observed in Ch or ACh content in N. accumbens, N. caudatus, N. interpeduncularis, locus coeruleus, septum, hippocampus and cortex. A decrease in CAT activity was found in the N. caudatus in agreement with earlier workers (McGeer et al., Exp. Geron. 6: 391, 1971). In other nuclei, CAT activity was unchanged.

These results clearly indicate that there is destruction of 5HT secreting neurons with aging. However, the cholinergic system is not uniformly affected during aging, which suggests that neuronal destruction is a process with a certain degree of selectivity. Probably neuronal destruction in aging reflects more of the exhaustion of a particular process which is typical of certain neuronal systems than a diffuse form of neuronal degradatio.

It is of interest to note that monoaminergic neurons and not cholinergic neurons are particularly vulnerable to aging, as indicated by our report, and by a decrease in the number of locue coeruleus neurons occurring in geriatric patients.

Further studies involving studies of the dynamics of the several transmitter systems would help elucidate how various synapses are modified during the aging processes.

Proposed course: This project is terminated.

Publications:

Cheney, D. L., Costa, E., Racagni, G. and Zsilla, G.: The cholinergic and adenylate cyclase systems in rat brain during development. In Brazier, M. A. B. and Coceani, F. (Eds.): Brain Dysfunction in Infantile Febrile Convulsions. New York, Raven Press, 1976, pp. 41-53.

Meek, J. L., Bertilsson, L., Cheney, D. L., Zsilla, G. and Costa, E.: Aging-induced changes in acetylcholine and serotonin content of discrete brain nuclei. J. Gerontol. 32: 129-131, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01505-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Anesthesia and acetylcholine turnover rate in rat brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. L. Cheney Chief, Section on Molecular Pharmacodynamics SMRP NIMH

COOPERATING UNITS (if any)
Dept. of Anesthesiology, College of Physicians and Surgeons of Columbia University, New York City, New York.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nitrous oxide increased the turnover rate of acetylcholine in diencephalon and septum and reduced the acetylcholine content in striatum. These effects were reversed by naltrexone suggesting that nitrous oxide releases or potentiates an endogenous analgesic or opioid.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the mechanisms that regulate the function of cholinergic neurons in the central nervous system.

Acetylcholine and choline concentrations and acetylcholine turnover rate were measured mass spectrometrically in different regions of rat brain during anesthesia with nitrous oxide. Thirty min after induction of inhalation of nitrous oxide (75%) and oxygen (25%) rats were infused for nine min with deuterated phosphorylcholine and killed by microwave radiation. Naltrexone (2 mg/kg; i.p.; 40 min) was administered in an attempt to block the effect of nitrous oxide on ACh turnover rate.

In preliminary experiments nitrous oxide reduced the concentration of ACh in striatum but not in other regions studied. This decrease was reversed by naltrexone pretreatment. The turnover rate of ACh was increased by nitrous oxide in diencephalon and septum but unchanged in cortex, striatum and hippocampus. Moreover, naltrexone reversed the nitrous oxide induced increase of the turnover rate or acetylcholine in septum and diencephalon.

Nitrous oxide has several features in common with the actions of opiates. It may be that nitrous oxide releases or potentiates an endogenous analgesic or opiate. A clarification of the influences exerted by various anesthetic agents on cholinergic systems may help in understanding the role that acetylcholine plays in consciousness.

The changes observed in diencephalon and septum will be studied in greater detail using slicing and punching techniques to localize the specific nuclei which are responsible for the increased ACh turnover. Moreover, the role of cholinergic mechanisms in sleep will be investigated.

Publications:

Ngai, S. H., Cheney, D. L. and Finck, A. D.: Effects of halothane, enflurane and ketamine on acetylcholine concentrations and turnover in rat brain structures. J. Anesthesiol., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01506-03 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Narcotic analgesics and the regulation of dopaminergic pathways of rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Gale	Staff Fellow	SMRP NIMH
Other:	A. Guidotti	Chief, Section on Neuro-endocrinology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analgesic doses of morphine and viminol R_2 increase striatal dopamine turnover rate but this increase does not appear related to either an increase in the firing rate of dopaminergic neurons or to the stimulation of postsynaptic dopaminergic receptors. However, morphine blocks the activation of striatal TH induced by neuroleptic drugs. Morphine, viminol R_2, azidomorphine increase cGMP in striatum and accumbens but not in cerebellum. The changes of cGMP in striatum and accumbens are blocked by naloxone. Thus activation of the cGMP system can represent the specific synaptic response following an interaction of the opiate receptor with its antagonist. The location of the opiate receptors and the relation of opiate receptor function with the DA neurons in substantia nigra and striatum is now under investigation.

PHS-6040

Project Description:

Our objective is to study the action of narcotic analgesics administered acutely and chronically on the function of the nigro-striatal system.

To establish if narcotic analgesics interact with the dopaminergic system at the pre- or postsynaptic receptor site we studied changes of catecholamine turnover rate, changes of tyrosine hydroxylase activity and changes of cAMP and cGMP concentrations in striatum. Analgesics doses of morphine and viminol R_2 increase the turnover rate of dopamine (DA) in rat striatum but fail to increase the striatal concentration of 3',5'-cyclic adenosine monophosphate (cAMP) or the affinity of tyrosine hydroxylase (TH) for the pteridine cofactor. When morphine is added to striatal homogenates it changes neither the basal activity of adenylate cyclase nor the enzyme activation by DA.

Since morphine interacting with its receptor in striatum does not increase cAMP, we wonder if morphine or its natural putative polypeptide transmitter could interact with the postsynaptic element producing an increase of cGMP. Morphine, azidomorphine, viminol R_2 injected in analgesic doses all increase cGMP in striatum and accumbens. This effect is blocked by naloxone but not by cholinergic or dopaminergic antagonists. The increase of cGMP in striatum is quite specific because these narcotic analgesics do not produce any increase of cGMP in cerebellum. Actually in this tissue there is a clear long lasting drop of cGMP concentration. Thus, the changes of cGMP in striatum following narcotics administration can be the consequence of an interaction of the opiate receptor with its agonist. Recently, we have observed that morphine injected systemically or intranigrally can block the haloperidol-induced activation of striatal TH. The activation of striatal TH induced by haloperidol is dependent by a blockade of postsynaptic DA receptors; morphine does not have any direct action on these receptors. It is, therefore, conceivable that opiate receptors present in striatum and nigra directly and indirectly regulate the function of DAergic neurons.

We are planning to study whether opiate receptors in striatum and nigra are located on DA neurons or they are on terminals of other neurons which release their transmitter in the vicinity of DA neurons.

The mechanism of narcotic dependence is relevant to the mission of NIMH in many ways. Our results show that analgesia is associated with an increase of striatal dopamine turnover but this increase of dopamine metabolism does not appear to be related to either an increase in the firing rate of dopaminergic neurons or to the stimulation of postsynaptic dopaminergic receptors. On

the other hand, morphine antagonizes the activation of striatal TH induced by haloperidol. This effect shows tolerance. Thus, a better understanding of the mechanisms involved may elucidate the basic mechanisms that control dopaminergic functions and the molecular basis of morphine tolerance and dependence.

Publications:

Racagni, G., Zsilla, G., Guidotti, A. and Costa, E.: Accumulation of cGMP in striatum of rats injected with narcotic analgesics: Antagonism by naltrexone. J. Pharm. Pharmacol. 28: 258-260, 1976.

Biggio, G., Guidotti, A. and Costa, E.: On the mechanism of the decrease in cerebellar cGMP content elicited by opiate receptor agonists. Naunyn Schmied. Arch. Pharmacol. 296: 117-121, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01507-07 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Possible neuronal alterations in muscular dystrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. L. Wood	Guest Worker	SMRP NIMH
Other:	D. L. Cheney	Chief, Section on Molecular Pharmacodynamics	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetically dystrophic mice (129 ReJ dy/dy) were found to possess a significantly lower brain acetylcholine content and turnover rate than littermate controls. Monoamine levels and turnover, however, were unaffected. These data suggest a central cholinergic defect which is presently being examined in various brain regions.

Project Description:

Our objective was to extend the original studies of whole brai which suggested a cholinergic defect in the dystrophic mouse as described in Project No. Z01 MH 01507-07 SMRP, 1976.

We are interested in examining this cholinergic defect in spec fic brain tracts and to further extend these studies to other models of muscular dystrophy to determine if this is a defect common to other dystrophic animals. The models being considered include the dystrophic hamster, chicken and various dystrophic mouse strains (129 ReJ, C57 Bl/6J dy^{2J}, C57 Bl/6J dy, and myo-dystrophy) of these models the C57 Bl/6J dy^{2J} strain is being investigated because of the lower cost and ready availability.

We are presently examining acetylcholine and choline concentra tions in several brain areas. Acetylcholine turnover is also being examined via the use of stable isotopes (Neuropharmacology 16: 25-30, 1977). These studies will hopefully pinpoint possible specific cholinergic tracts which are abnormal in dystrophic ani-mals and thereby form the basis for the development of rational therapy in human muscular dystrophy.

We further propose to investigate the development of this c linergic defect and to examine cholinergic transmission in nerve muscle preparations, in vitro, for dystrophic and normal litter-mates animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01508-07 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Turnover of acetylcholine and opiate action, dependence and tolerance.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Moroni	Visiting Associate	SMRP NIMH
Other:	D. L. Cheney	Chief, Section on Molecular Pharmacodynamics	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1.) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In rats, intraventricular injection of morphine and beta-endorphin decrease the turnover rate of acetylcholine (TR_{ACh}) in the cortex, hippocampus, N. accumbens and globus pallidus, but not in the striatum. Beta-endorphin is approximately 100 times more active than morphine both in inducing analgesia and catalepsy and in decreasing the TR_{ACh}. Naltrexone, a narcotic antagonist reverses the action of beta-endorphin and morphine. Intraseptal injections of morphine and beta-endorphin are not able to induce analgesia or catalepsy, but decreases the TR_{ACh} in the hippocampus. Intraseptal injections of naltrexone curtail the decrease of hippocampal TR_{ACh} caused by intraperitoneal morphine.

These results suggest that only certain cholinergic neurons are regulated by opiate receptors. Specifically they demonstrate that the septal hippocampal cholinergic pathway is modulated by opioid receptors and that this action is dissociated from the onset of analgesia.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective has been to measure the acetylcholine (ACh) turnover rate (TR_{ACh}) in various brain nuclei after injection of morphine or of opioid peptides (alpha- or beta-endorphin) into the ventricles or in selected brain areas. In this way, it is possible to better understand the site(s) of action of morphine and of endogenous peptides with opiate like activity and the eventual involvement of cholinergic mechanisms in some of the actions of narcotics.

The procedures and the techniques for calculating TR_{ACh} have been described in detail in Project Z01 MH 01509-06 SMRP, 1975, and Z01 MH 01503-02 SMRP, 1975. Analgesia was measured using the tail flick method of D'Amour and Smith (JPET 72: 74-79, 1941) as modified by Harris and Pierson (JPET 143: 141-148, 1964), catalepsy was measured according to Costall and Olley (Neuropharmacology 10: 207-306, 1961). The polyethylene cannulae were implanted into the rat's ventricles according to Robinson et al. (Physiol. Behav. 4: 123-124, 1969) and other cannulae (stainless steel, 22 gauge, Plastic Product Co., Roanoke, Va.) were stereotaxically implanted into the septum following the coodinates reported in the König and Klippel atlas (The Rat Brain, 1963). In the experiments in which the animals with intraventricular cannulae were used, morphine, alpha-endorphin or beta-endorphin were injected in a volume of 10 µl of saline. In the animals with cannulae implanted in specific brain nuclei, the opioids were injected in a volume of 1 µl with the aid of a gear-driven Hamilton syringe. At various times after the injections, analgesia, catalepsy and TR_{ACh} were measured. For the measurement of TR_{ACh}, the animals were killed by a beam of microwave radiation focussed on their skull. The brains were removed, mounted on a microtome stage and sliced in 400 µ thick coronal sections. Various nuclei were punched out with stainless steel tubing (0.8-1.2 mm). The punches were processed and finally injected into a GC-MS for the determination of endogenous and deuterated choline and ACh.

Intraventricular injections of beta-endorphin were found to have the ability to induce analgesia and catalepsy as well as a dose dependent decrease of the TR_{ACh} in the cortex, hippocampus, N. accumbens and globus pallidus. No change was observed in the N. caudatus. These effects were qualitatively similar to those induced by intraventricular morphine which was approximately 30 times less active. Alpha-endorphin at a dose 30 times higher than the minimal effective dose of beta-endorphin was completely inactive. These studies suggest that only certain cholinergic neurons are regulated by opiate receptors. They also support the hypothesis that the "opioid peptides" are inhibitory neurotransmitters. In the experiments described above we were impressed by the fact that the TR_{ACh} in the hippocampus was inhibited

85% after intraventricular beta-endorphin. As the hippocampus contains a low density of opioid receptors, we decided to further investigate this phenomenon. This investigation was undertaken also because the decrease in hippocampal TR_{ACh} induced by narcotics correlates well with the onset of narcotic induced analgesia (Zsilla, JPET 199: 662-668, 1976).

By using intraseptal injections, it was possible to dissociate the decrease in the hippocampal TR_{ACh} from the onset of analgesia. From these investigations we can conclude that the effect of opioids on the TR_{ACh} in the hippocampus is due to an action on the septal neurons, and is not correlated with analgesia but with some of the other numerous actions of the opioids.

From a biomedical point of view, it is possible that the effect of opioids on the TR_{ACh} in hippocampus may be correlated with the subjectively pleasant effect of the opioid which is an important link in the craving phenomenon and, eventually, in the development of narcotic dependence. The action of opiate and opioid peptides on the N. accumbens and in the N. pallidus may, on the other hand, be correlated with the opiate induced catalepsy. It is possible to verify these hypotheses by chronic treatment with morphine and/or other opiate agonists. In fact, while tolerance develops to the analgesic and cataleptic (G. pallidus, N. accumbens) effects of these drugs, tolerance develops to a much lesser degree to the subjective effect (hippocampus?).

We plan to study the TR_{ACh} during the development of tolerance and physical dependence and try to correlate the actions of opiate and opioid peptides on the TR_{ACh} with the behavioral effects of these drugs. Furthermore, we plan to investigate possible biochemical differences among the opiate drugs and the naturally occurring opioid peptides.

Publications:

Zsilla, G., Cheney, D. L., Racagni, G. and Costa, E.: Correlation between analgesia and the decrease of acetylcholine turnover rate in cortex and hippocampus elicited by morphine, myseridine, viminol R_2 and azidomorphine. J. Pharmacol. Exp. Ther. 199: 667-668, 1976.

Zsilla, G., Racagni, G., Cheney, D. L. and Costa, E.: Constant rate infusion of deuterated phosphorylcholine to measure the effects of morphine on acetylcholine turnover rate in specific nuclei of rat brain. Neuropharmacology 16: 25-30, 1977.

Zsilla, G., Cheney, D. L. and Costa, E.: Acetylcholine turnover rate in specific brain nuclei: Effects of narcotic analgetics. In Manara, L. and Simon, E. S. (Eds.): Factors Affecting the Actions of Narcotics, 1977.

Moroni, F., Cheney, D. L. and Costa, E.: Beta-endorphin inhibition of ACh turnover in nuclei of rat brain. Nature, in press, 1977.

Moroni, F., Cheney, D. L. and Costa, E.: Inhibition of acetylcholine turnover rate in hippocampus by intraseptal injection of β-endorphin and morpine. Naunyn-Schmied. Arch. Pharmacol., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01509-07 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacologicla studies of acetylcholine turnover: peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Malthe-Sorenssen	Guest Scientist	SMRP NIMH
Other:	D. L. Cheney	Acting Chief, Section on Molecular Pharmacodynamics	SMRP NIMH
	P. Wood	Guest Scientist	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.85	1.65	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The peptide substance P decreased the turnover rate of acetylcholine in hippocampus and septum without changing the levels of either acetylcholine or choline.

Somatostatin, neurotensin and TRH, all pituitary neurohormones, interfered with the cholinergic nervous system. All three peptides enhanced the TRACh in some brain areas, but the profile of activity differed for different peptides, indicating a specific role in neuronal regulation.

Angiotensin II given intraventricularly to rats, increased the blood pressure and induced drinking behavior in accordance with previous observations. In addition angiogensin II decreased specifically the level of acetylcholine in cortex, but did not change the turnover rate under the conditions used. The present results suggest the presence of specific receptor sites for peptides in different parts of the brain, which play an important role in regulation of cholinergic neurons.

Project Description:

The object of this study was to investigate the effect of neuronally active peptides on central cholinergic mechanisms by measuring the in vivo turnover rates of acetylcholine stores in cholinergic neurons. The turnover rate was measured by applying principles of steady state kinetics. Substantial indirect evidence has previously demonstrated that measurements of acetylcholine turnover rates are of value in understanding the functional participation of a specific group of cholinergic neurons during behavioral activities or in the mode of action of neuromodulators and drugs.

The methodology and techniques used for measuring the turnover rate in this project were described in Project No. Z01 MH 01509-07 SMRP 1976. Six different peptides were injected intraventricularly in rats. Substance P, a putative neurotransmitter; three pituitary hormones, TRH, somatostatin and neurotensin; and angiotensin I and II, peptides active in regulating hypertension.

The rats were all canulated by a polypropylene tube into the ventricle and the peptides were injected in a volume not exceeding 10 µl. The animals were killed by focussing a beam of microwave radiation directly on the skull. The brains were removed, dissected and processed as previously described.

Our data suggested that by measuring the turnover rate of acetylcholine it was possible to detect a modulatory role on the metabolism of acetylcholine in cholinergic neurons mediated by activation of receptors, specific for certain putative neuromodulators.

Substance P, a peptide acting as a putative neurotransmitter in several neuronal circuits, decreased the turnover rate of acetylcholine in hippocampus without changing the levels of acetylcholine. The turnover rate was not changed in striatum, cortex, diencephalon or medulla. Since it has been shown that there is little or no substance P in hippocampus, the inhibition elicited by substance P must reflect a presynaptic depolarization by this peptide of either terminals of excitatory neurons impinging on cholinergic cell bodies or cell bodies of inhibitory neurons impinging on septal cholinergic neurons. In addition it was demonstrated that substance P had a slight analgesic effect when injected into the periaquaductal grey (dorsal raphe).

Apart from their hypophysiotropic effect TRH, somatostatin and neurotensin exerted profound effects on central cholinergic neurons. TRH increased the turnover rate of acetylcholine in the cortex whereas neurotensin and somatostatin did not. All peptides failed to change the turnover rate in striatum. Ne

tensin and somatostatin increased the turnover rate of acetylcholine in diencephalon, whereas only somatostatin increased the turnover rate in pons medulla. At high doses somatostatin induced a turning behavior. This behavioral effect was followed by an increase in the turnover rate of acetylcholine in hippocampus, which could not be detected at lower doses.

Angiotensin II, which participates in the renin-angiotensin system for regulation of hypertensin and as a dipsogenic drug, decreased the levels of acetylcholine in cortex, but failed to change the turnover rate. No other change could be detected.

A variety of evidence suggests that hypothalamic hormones excerts non pituitary brain effects with behavioral and psychotic consequences and to understand the mechanism underlying these effects is of great importance. The nonuniform distribution of the peptides studied and their ability to specifically modulate the activity of cholinergic neurons in several brain nuclei may be indicative of their involvement in modulating behavioral and physiological parameters through cholinergic mechanisms.

We will continue our studies on the regulation of cholinergic mechanisms by measuring the turnover rate and the effect of neuromodulators on these mechanisms to gain further insight into the peptidergic regulation of cholinergic function.

Publications:

Cheney, D. L., Moroni, F., Malthe-Sorenssen, D. and Costa, E.: Endogenous modulators of acetylcholine turnover rate. In Jenden, D. J. (Ed.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01510-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Interaction of GABA-ergic and cholinergic dynamics: Effect of marijuana

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Revuelta	Visiting Associate	SMRP NIMH
Other:	F. Moroni	Visiting Scientist	SMRP NIMH
	D. L. Cheney	Chief, Section on Molecular Pharmacodynamics	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Δ9-THC decreases the TRACh in hippocampus and striatum but not in cortex or diencephalon. GABA mimetics, muscimol and diazepam decrease the TRACh in cortex and diencephalon without altering the TRACh in hippocampus or striatum. These results suggest that Δ9-THC does not inhibit the cholinergic mechanisms in hippocampus through an action on GABA neurons. Δ9-THC is only one of the active components in the "cannabis sativa". Other active principles such as the cannabidiols possess anti-convulsant activity. It is possible that these compounds act by modifying the GABAergic system.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this research was the study of the effects of Δ9-THC (one of the active components of cannabis sativa) on the TR_{ACh} and on the TR_{GABA}.

The techniques and methods used in this study were described in the Project No. Z01 MH 01509-08 SMRP, 1976. Male Sprague-Dawley rats were injected with muscimol (9 μmoles/kg; i.v.) a GABA receptor agonist or diazepam (10 μmoles/kg; i.p.) thought to indirectly activate GABA receptors and with various dose of delta 9-THC.

Δ9-THC (5 mg/kg; i.v.) was found to decrease the TR_{ACh} in the hippocampus and striatum leaving the TR_{ACh} in cortex, brainstem and diencephalon unchanged. On the contrary, muscimol and diazepam decreased the turnover rate of ACh in the brainstem and cortex but failed to change the TR in striatum or hippocampus. Since striatum contains an abundance of GABA neurons and intrinsic cholinergic neurons, it maybe inferred that the metabolism of ACh and presumably the activity rates of striatal cholinergic neurons are not regulated by the activation of GABA receptors. Similar considerations apply to the cholinergic pathway projecting from septum to hippocampus. Thus the effect of Δ9-THC on the TR_{ACh} in striatum and hippocampus does not appear to be mediated through an action on GABA receptors.

From the biomedical point of view that investigation can help to better understand the hallucinogenic effect of Δ9-THC and other active components of the widely used "cannabis sativa". Moreover, this research can help to clarify the interrelationship between the cholinergic and GABA-ergic systems.

We plan to study the TR_{ACh} and the TR_{GABA} after various doses of Δ9-THC and of other active components of the "cannabis sativa" such as the cannabidiols, which has anticonvulsant activity. We plan also to investigate if "tolerance" develops to these biochemical effects of the cannabinols.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01511-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Neuronal monoamines: A study of their integration and regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Gale	Staff Fellow	SMRP NIMH
Other:	A. Guidotti	Chief, Section on Neuroendocrinology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C.20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tyrosine hydroxylase (TH) from rat neostriatum shows an increase in affinity for pteridine cofactors after administration of antipsychotic drugs. This correlates with an increased firing rate of nigroneostriatal dopamine (DA) neurons and an increase in biosynthesis of DA in neostriatum. We have found that discrete lesions of neuronal pathways descending from striatum to substantia nigra (SN) abolishes the ability of antipsychotics to cause activation of striatal TH. These lesions resulted in a 60-90% decrease of GABA and substance P in SN without damaging DA containing neurons. Application of GABA-mimetic compounds such as muscimol or diazepam, either systemically or directly into SN, blocked the antipsychotic-induced activation of striatal TH. On the other hand, systemic administration of GABA antagonists such as picrotoxin or isoniazid potentiated the action of haloperidol. The data suggest that GABA terminals in SN, and possibly also substance P terminals in SN, play a role in the mediation of the effect of antipsychotic drugs on the activity of nigrostriatal DA neurons.

Project Description:

Catecholaminergic nerve function is associated with the metabolic status of the amine which the neuron uses as a transmitter. In noradrenergic nerves, regulation of this metabolism involves the biosynthesis or activation of the enzyme molecules which catalyze the rate-limiting processes for norepinephrine formation. Since the regulation of the synthesis of brain dopamine is poorly understood, we attempted to study the mechanisms involved in the regulation of tyrosine hydroxylase in dopaminergic nerve terminals. Our immediate objectives were:

1) To measure short-term changes in the activity of tyrosine hydroxylase in striatum and nucleus accumbens after treatment with drugs that specifically impair dopaminergic transmission.

2) To characterize the changes of tyrosine hydroxylase activity induced by drugs.

3) To investigate other brain neuronal systems (GABA, polypeptides, acetylcholine) that might be involved in the regulation of tyrosine hydroxylase in dopaminergic neurons.

We made the following observations:

1) A single intraperitoneal injection of an antipsychotic drug (methiothepin, haloperidol, pimozide, chlorpromazine, clozapine and thioridazine) produces a decrease of the apparent Km of tyrosine hydroxylase for the pteridine cofactor in striatum and nucleus accumbens. The doses of methiothepin, pimozide and haloperidol required to change the kinetic state of tyrosine hydroxylase in striatum were lower than those required to produce similar changes in nucleus accumbens. In contrast, clozapine and thioridazine were more effective in nucleus accumbens. Chlorpromazine was equally potent in these two brain regions. Haloperidol increased the turnover rate of dopamine in striatum with doses that were relatively lower than those required for nucleus accumbens. In contrast, clozapine was more active in increasing turnover rate of dopamine in nucleus accumbens, while chlorpromaine was equally potent in both regions.

Thus, antipsychotics with a high incidence of extrapyramidal side effects preferentially increase dopamine turnover and change tyrosine hydroxylase in striatum.

2) Discrete electrolytic lesions placed anterior and lateral to substantia nigra resulted in a 60-90% decrease in nigral GABA and substance P, without a significant loss of nigral or striatal tyrosine hydroxylase. These lesions prevented the activation of

striatal TH by haloperidol when administered 7-10 days postoperatively. This suggests that the integrity of the nigrostriatal DA neurons alone is insufficient for the haloperidol-induced activation and that striato-nigral pathways (GABA and/or substance P) are required for the effect of antipsychotics on DA neuronal function.

Using a series of hemitransections as well as kainic acid-induced lesions of neuronal cell bodies in striatum, we were able to demonstrate that the striatonigral projections containing substance P and GABA were respectively independent. This raises the possibility that striatal influence on SN DA neurons is mediated by at least two separate neurotransmitter systems projecting to SN.

3) The electrolytic lesions described above also resulted in a virtually complete loss of DA-sensitive adenylate cyclase in SN. On the other hand, treatment with 6-hydroxydopamine, which selectively and almost completely (80%) destroyed nigral DA neurons, did not result in a loss of DA-sensitive adenylate cyclase in SN. These data demonstrate that DA receptors associated with adenylate cyclase, are located on terminals of non-DA neurons in the SN. Such DA receptors constitute a possible site of influence of drugs affecting DA transmission.

4) A role for GABA was indicated by the fact that systemically administered GABA mimetic compounds (muscimol, diazepam) blocked the action of haloperidol on striatal TH, and GABA antagonists (picrotoxin, isoniazid) potentiated haloperidol. At least one important site of action of GABA activity was shown to be in SN, since local application of muscimol (via intracerebral chronic cannula) antagonized the ability of haloperidol to activate striatal TH. The action of muscimol was reversed by the GABA receptor antagonist bicuculline.

5) Haloperidol or the DA agonist, apomorphine, when placed directly in SN failed to affect the kinetic state of striatal TH and also failed to change the ability of systemically administered haloperidol to activate striatal TH suggesting that the DA receptors in SN do not constitute a critical site for the action of haloperidol on striatal TH.

An increasing body of evidence has been accumulated suggesting that brain monoaminergic function may be altered in mental disorders. The beneficial effects of antipsychotic drugs in the treatment of schizophrenia indicate that brain dopaminergic neurons might be involved in the etiology or at least in the manifestation of some symptoms of this disease. An understanding of the mechanisms whereby antipsychotic drugs affect synaptic function of central dopaminergic neurons appears to be a reasonable approach to study the biochemical basis of schizophrenia. More-

over, the study of the effects of antipsychotic drugs on dopaminergic mechanisms may offer a better understanding of the regulation of such neuronal systems. The finding reported here may have a practical application in designing new antipsychotic drugs which would affect specifically neuronal systems such as GABA such as GABA that regulate dopaminergic function.

In order to determine more precisely the role of striatonigral pathways in the regulation of DA neuronal function, we plan to distinguish the respective influence of nigral substance P and GABA on the changes induced by antipsychotic administration. We also plan to determine the role of other neuromodulators in the SN and striatum, such as opiate peptides, for the regulation of DA activity.

Since antipsychotics are used chronically in clinical conditions, it is important to understand what biochemical changes occur after long-term administration. Using TH, GABA, adenylate cyclase, and dopamine turnover (in vivo) as indexes of the functioning of different aspects of neural function, we expect to determine how the various components change or adjust to the prolonged influence of antipsychotic treatment.

Publications:

Gale, K., Guidotti, A. and Costa, E.: On the location of dopamine-sensitive adenylate cyclase in substantia nigra. Science 195: 503-505, 1977.

Gale, K. and Guidotti, A.: GABA mediated control of rat neostriatal tyrosine hydroxylase revealed by use of intranigral muscimol. Nature 263: 691-693, 1976.

Guidotti, A., Gale, K., Toffano, G. and Costa, E.: Regulatory role of GABA neurons on the activation of striatal tyrosine hydroxylase by haloperidol. In Weiner N. and Usden, E. (Eds.): Biochemistry and Function of Monoamines Enzymes. New York, Marcel Dekker, in press, 1977.

Guidotti, A. and Gale, K.: Participation of GABA receptors in the short-term activation of striatal tyrosine 3-monooxygenase elicited by neuroleptics. In Costa, E. and Gessa, G. L. (Eds.): Advances in Biochemical Psychopharmacology. Vol. 16, New York, Raven Press, 1977, pp. 455-460.

Guidotti, A., Gale, K., Hong, J. and Toffano, G.: Models to study drug effects on the integrated function of GABAergic, peptidergic (substance P) and dopaminergic neurons. In Proceedings of the symposium entitled: Interactions Among Putative Neurotrans

mitters in Brain. New York, Raven Press, in press, 1977.

Gale, K., Costa, E., Hong, J., Toffano, G. and Guidotti, A.: Evidence for a role of nigral GABA and substance P in the haloperidol induced activation of striatal tyrosine hydroxylase. J. Pharmacol. Exp. Ther., in press, 1977.

Gale, K., Hong, J. and Guidotti, A.: Presence of substance P and GABA in separate striatonigral neurons. Brain Res., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01512-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The role of cyclic nucleotides in the regulation of pituitary function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Grandison	Staff Fellow	SMRP NIMH
Other:	A. Guidotti	Chief, Section on Neuro-endocrinology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

cAMP content, phosphodiesterase activator and protein kinase activity can be increased in the anterior pituitary of rats following intraperitoneal injection of reserpine, isoniazid or beta-endorphin. The effects of reserpine can be blocked by pargyline (a monoamine oxidase inhibitor). The effect of isoniazid is not abolished by pargyline but is abolished by small doses of muscimol, diazepam and nipecotic acid, all drugs that increase the GABAergic function. Muscimol, diazepam and nipecotic acid are ineffective against reserpine. The effects of beta-endorphin are blocked by naloxone. These experiments suggest that noradrenaline, GABA and endorphin independently participate in hypothalamic regulation of pituitary function.

PHS-6040
(Rev. 10-76)

Project Description:

Neuronal mechanisms trans-synaptically regulate the synthesis and release of polypeptide hormones from hypothalamus. These polypeptides in turn regulate pituitary function. The release of hormones from anterior pituitary is associated with an increase of pituitary cAMP content presumably elicited by hypothalamic releasing factors. In this case the effects of drugs on different hypothalamic neurotransmitter systems were monitored by in vivo measurement of pituitary cAMP.

The depletion of hypothalamic biogenic amines induced by reserpine (10 mg/kg, i.p.) resulted in a 4-fold increase in anterior pituitary cAMP. Administration of pargyline prior to reserpine blocked the increase of cAMP. Depletion of hypothalamic GABA by subcutaneous (400 mg/kg, s.c.) or intrahypothalamic (500 µg) injection of isoniazid was associated with a 3 to 10 fold increase in cAMP in both the anterior and posterior pituitary. Maximal stimulation occurred by 40 minutes with a return to basal levels by 120 minutes. Prior intrahypothalamic administration of muscimol, a GABA receptor agonist with an affinity for hypothalamic GABA receptors greater than that of GABA, prevented cAMP accumulation elicited by isoniazid. In contrast, the cAMP accumulation following reserpine was not blocked by muscimol while the increase after isoniazid was not diminished by pargyline. In addition, intraventricular injection of 10 µg of beta-endorphin, which is known to stimulate prolactin and growth hormone release, increased anterior pituitary cAMP 2 to 3 fold. Intrahypothalamic injection of beta-endorphin produced cAMP accumulation at 20 min but by 40 minutes cAMP returned to basal levels. No change in cAMP was observed after injection of 10 µg of alpha-endorphin. Naltrexone blocked the accumulation of cAMP induced by beta-endorphin but had no effect given alone. These data suggest that catecholamines, GABA and beta-endorphin are all involved in hypothalamic regulation of anterior pituitary function.

Since cAMP and cGMP function as second messengers in neuroendocrine responses of pituitary, we have measured factors that express their activity at the molecular level (activation of protein kinase) or control their concentration at the site of action (phosphodiesterase activator). We have shown that the increase of cAMP in pituitary following reserpine injection or exposure of the rat to cold is controlled by the activity of a phosphodiesterase activator. The concentration of this activator also can be measured in different hypothalamic nuclei and can be used as a parameter to detect activation of receptors. The other parameter which can be measured in hypothalamic nuclei and other brain nuclei with great precision and sensitivity is the activity of the cAMP-independent and cAMP-dependent protein kinase.

Reserpine, chlorpromazine, isoniazid and cold exposure activate protein kinase of pituitary. The time course of activation lasts longer than the increase of pituitary cAMP. In fact, two hours after the injection of reserpine and the termination of cold exposure the levels of cAMP approach normal values but protein kinase is still fully activated.

In preliminary experiments measuring hormone release from the pituitary we have established that GABA-, opiate- and DA-receptors control the release of different types of hormones. While the decrease of GABA receptor function resulted in an increase release of ACTH, no changes of prolactin release were observed. In contrast, stimulation of GABA or opioid receptors resulted in the release of prolactin. After blockade of DA receptors, both corticosteroids and prolactin are released from anterior pituitary.

Since we have established that different neurotransmitter systems (GABA, DA, peptide containing neurons) in hypothalamus affect hormone release, cAMP levels, protein kinase activity and phosphodiesterase activator, these biochemical parameters can be used to locate the neuronal pathway involved in the elaboration of the response of the organism to various environmental stimuli and drugs.

We also plan to evaluate whether the activation of protein kinases results in phosphorylation of the nuclear proteins mediating trans-synaptically changes in synthesis of polypeptide releasing factors or polypeptides hormones.

Publications:

Costa, E., Guidotti, A., Uzunov, P. and Zivkovic, B.: Methods to study the in vivo regulation of cyclic nucleotides in pituitary. In Kumar, T. C. and Karger, J. (Eds.): Neuroendocrinology Regulation of Fertility. Switzerland, Basel, 1976, pp. 196-205.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01513-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Synthesis of enzymes that regulate rate-limiting processes in control of the neuronal tissue.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Hanbauer	Pharmacologist	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

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Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of these studies was to elucidate the participation of hormones and the second messengers cyclic 3',5'-adenosine monophosphate (cAMP) or cyclic 3',5' guanosine monophosphate (cGMP) in the regulation of tyrosine hydroxylase (TH) biosynthesis in tissues containing cells that synthesize catecholamines. Previous studies had shown that at least 3 types of receptors (β-adrenergic, nicotinic and corticosterone receptors) were involved in the induction of tyrosine hydroxylase in superior cervical ganglion. In the carotid body the induction of TH can involve corticosterone, muscarinic and oxygen receptors. The participation in the induction of a Ca^{2+} dependent activator protein is supported by the present experiments.

PHS-6040
(Rev. 10-76)

Project Description:

The exposure of rats to a confining atmosphere containing 5% O_2 for two 30-min periods separated by a 20-min period of 20% O_2 causes an increase in the V_{max} of TH at 24 and 48 hr after stimulus application. Exposure to hypoxic conditions for only 30 mi(failed to change TH activity in the carotid body. The increase in V_{max} depends upon protein synthesis and can be abolished by transection of carotid sinus nerve or by a treatment with 3-acetylpyridine. The Km of the carotid body TH for 6-methyl-5,6,7,8 tetrahydropterin remains unchanged after exposure to hypoxic conditions. The induction of TH in the carotid body caused by hypoxia is tissue specific. TH is not induced in adrenal medulla, carotid artery or superior cervical ganglia of rats exposed to hypoxia. Moreover, hypoxia fails to increase dopamine-β-hydroxylase or choline acetyltransferase activity in the carotid body.

Injection of dexamethasone also induce TH as does injection of methacholine but not of incative. Hypoxia, dexamethasone and methcholine could not be studied in their efficiency as stimuli to increase cAMP content because of the inherent technical difficulties. However we have measured the content of Ca^{2+} binding activator protein in membranes. The stimuli cause a release of the Ca^{2+} binding protein which, indirectly can be taken as an evidence of the second messenger response.

We plan to develop methods to measure more directly the Ca^{2+} binding protein content in membranes.

Publications:

Hanbauer, I., Kopin, I. J., Guidotti, A. and Costa, E.: Induction of tyrosine hydroxylase by beta-adrenergic receptor agonists in normal and decentralized sympathetic ganglia. Role of 3',5' cyclic adenosine monophosphate. J. Pharmacol. Exp. Ther. 193: 95-104, 1975.

Hanbauer, I. and Guidotti, A.: cAMP dependent regulation of tyrosine 3-monooxygenase in adrenal medulla: Effect of denervatio Naunyn Schmied. Arch. Pharmacol. 287: 213-217, 1975.

Hanbauer, I., Lovenberg, W., Guidotti, A. and Costa, E.: R of cholinergic and glucocorticoid receptors in the tyrosine hydroxylase induction elicited by reserpine in superior cervical ganglion. Brain Res. 96: 197-200, 1975.

Hanbauer, I., Costa, E. and Guidotti, A.: Trans-synaptic induction of tyrosine hydroxylase in superior cervical ganglia: Participation of post-synaptic and steroid receptor. In Chemical

Tools in Catecholamine Records. Vol. 2, New York, North Holland, 1975, pp. 175-182.

Hanbauer, I., Lovenberg, W. and Costa, E.: Induction of tyrosine 3-monooxygenase in carotid body of rats exposed to hypoxic conditions. Neuropharmacology 16: 277-282, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01514-05 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Trans-synaptic control of protein synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief, Section on Neuroendocrinology	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Preclinical Pharmacology

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In chromaffin cells of adrenal, the sequence of molecular events whereby the genetic code expression is regulated trans-synaptically includes an increase in the cAMP/cGMP concentration ratio, an activation of cytosol cAMP-dependent protein kinase and the translocation of the low molecular weight catalytic subunit from the cytosol to the subcellular fraction. The protein kinase of nuclei is not regulated by cAMP but it increases during the trans-synaptic induction of tyrosine-3-monooxygenase (TH) as a result of a translocation of the protein kinase catalytic subunits from cytosol. Transection of the splanchnic nerve after the increase of cAMP elicited by reserpine has occurred and the cytosol protein kinase has been activated and translocated reverts both phenomena and prevents the trans-synaptic induction of TH. Thus, the activation and translocation of protein kinase, triggered by the initial increase of cAMP acts as a long range messenger for the trans-synaptic regulation of the genetic code expression in adrenal medulla.

Project Description:

Our objective was to study the regulation of the synthesis of specific proteins by trans-synaptic mechanisms or by the injections of cholinergic receptor agonists.

An increase of cAMP/cGMP concentration ratio is a stimulus-coupled biochemical event occurring in adrenal medulla of rats during direct or indirect stimulation of medullary nicotinic receptors. When this second messenger response lasts at least 60 minutes it elicits a delayed increase in protein synthesis including that of TH molecules. When this induction depends on the indirect stimulation of nicotinic receptors due to the release of acetylcholine from nerve terminals, it has been termed trans-synaptic.

We studied with great detail this long-range cascade of macromolecular changes elicited trans-synaptically in the enterochromaffin cells of rat adrenal medulla because we thought it could serve as a model of the macromolecular mechanisms mediating communication between activation of membrane bound transmitter receptors and nucleus. Probably it is critical for the induction that the increase in the cAMP content, elicited by the sustained activation of nicotinic receptors lasts at least 1 hr because catalytic subunits of the cAMP-dependent protein kinase must remain dissociated long enough to transfer in appropriate numbers from cytosol into the nuclei. In fact a dissociation of catalytic subunits of PK lasting 2 to 3 hrs appears to be an obligator event that mediates the increase in TH synthesis because about 30 to 40% of the cytosolic catalytic subunits of the cAMP-dependent type I protein kinase must be transferred (translocated) tc the nucleus and other subcellular particles. This nuclear trans location of the enyzme catalytic subunit persists longer than 1(hours and is not paralleled by a proportional translocation of the regulatory subunits of the same enzyme.

Probably, this translocation process requires an integrity o cholinergic input to the chromaffin cells because in denervated adrenal medulla, stimuli, such as treatment with aminophylline which increases cAMP and activates the cytosol cAMP-dependent protein kinase (PK) and no longer produces the translocation. Whenever translocation lasts less than 10 hrs, TH fails to be duced.

These and other results suggest that the activation of cAMP-dependent PK and its translocation from cytosol to subcellular structures are obligatory intermediary processes triggered by he early increase of cAMP. We are now investigating the mechanis by which these processes selectively can regulate the expressi of the genetic code during the induction of TH trans-synapti

elicited in chromaffin cells.

Since in adrenal medulla the availability of catecholamines and the adrenergic function are regulated by tyrosine hydroxylase and since adrenergic mechanisms have been implicated in the etiology of affective disorders, an understanding of the molecular nature of the regulation of catecholaminergic biosynthesis may open new avenues in the therapy of mental diseases.

The goal of our future research is to study the molecular mechanism that in the chromatin structure of neuronal cells regulates the transcription of individual genes after translocation of catalytic subunits of protein kinase from cytosol to the nucleus.

Publications:

Costa, E., Kurosawa, A. and Guidotti, A.: Activation of nuclear translocation of histone kinase during the trans-synaptic induction of tyrosine-3-monooxygenase. Proc. Nat. Acad. Sci. USA 73: 1058-1062, 1976.

Kurosawa, A., Guidotti, A. and Costa, E.: Induction of tyrosine 3-monooxygenase elicited by carbamylcholine in intact and denervated adrenal medulla: Role of histone kinase activation and translocation. Mol. Pharmacol. 12: 420-423, 1976.

Kurosawa, A., Guidotti, A. and Costa, E.: Induction of tyrosine 3-monooxygenase in adrenal medulla: Role of protein kinase activation and translocation. Science 193: 691-693, 1976.

Guidotti, A., Kurosawa, A. and Costa, E.: Role of histone kinase activation and translocation in the mediation of the transsynaptic induction of tyrosine hydroxylase in rat adrenal medulla. In Costa, E., Giacobini, E. and Paoletti, R. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1976, pp. 455-474.

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Guidotti, A. and Costa, E.: Commentary on transsynaptic regulation of tyrosine 3-monooxygenase biosynthesis in rat adrenal medulla. Biochem. Pharmacol., in press, 1977.

Guidotti, A. and Costa, E.: The induction of tyrosine 3-monooxygenase in rat adrenal medulla: A model for the transsynaptic regulation of gene expression. In Neuropharmacology and Behavior. New York, Plenum Press, in press, 1977.

Costa, E. and Guidotti, A.: Adrenal medulla: A model to stu the transsynaptic regulation of gene expression. In Weiner, N. and Usdin, E. (Eds.): Proceedings of the Symposium on Biochemistry and Function of Monoamine Enzymes. New York, Marcel Dekker, in press, 1977.

Costa, E. and Guidotti, A.: Molecular mechanisms mediating the transsynaptic regulation of gene expression in adrenal medulla. In Lipton, M. and DiMascio, A. (Eds.): Psychopharmacology: A Generation of Progress. New York, Raven Press, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01515-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of cerebellar cGMP and cAMP by excitatory or inhibitory transmitters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Bandle	Visiting Associate	SMRP NIMH
Other:	A. Guidotti	Chief, Section on Neuroendocrinology	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Increase of cerebellar cGMP content without changes of cAMP were measured after chemical stimulation of the climbing or mossy fiber systems. Lesion of the climbing fiber system with 3-acetylpyridine blocks the increase of cGMP produced by harmaline (a selective activator of the olivo-cerebellar pathway) but leaves unabated the increase of cGMP produced by glutamic acid (a exitatory putative amino acid transmitter) or by isoniazid (a drug that decreases GABA levels). Apomorphine increases cerebellar cGMP and haloperidol or morphine decreases cerebellar cGMP when injected in striatum but not in cerebellum, whereas, diazepam decreases cerebellar cGMP when injected in cerebellum but not in striatum. These results suggest that two exitatory transmitters released by the parallel or climbing fibers and GABA released by cerebellar interneurons are independently involved in changing cGMP content on the molecular layer of cerebellum. The molecular mechanism of the message mediated by the increase of cGMP is presently being studied.

Project Description:

The cerebellum is the brain region where the functional synaptology of various neurons has been determined with the greatest detail and precision. The project is designed to relate changes of cAMP and cGMP with the release of transmitters in the cerebellar cortex and deep cerebellar nuclei. Such studies are possible in cerebellum because:

1) The climbing fibers and the mossy fibers are the two major exitatory inputs to the Purkinje cells.

2) The function of Purkinje cells is regulated by inhibitory (GABAergic) interneurons.

3) The Purkinje cells are the sole output from the cerebellum and release GABA on the deep cerebellar neurons.

Previous reports have shown that pharmacological manipulations of climbing or mossy fibers not only change the firing pattern of Purkinje cells but also increase the cGMP content of the cerebellar cortex. Similarly, manipulation of the cerebella GABAergic function changes the firing rate of the Purkinje cells and the cerebellar cGMP content. When the concentration of cerebellar GABA is increased or GABA receptors are stimulated, the rate of Purkinje cells firing and the cGMP content of cerebellar cortex are reduced. Conversely, when GABA receptors are blocked, the rate of Purkinje cell firing and the cerebellar cGMP content is increased.

Experiments were then directed to elucidate the possible role of the climbing and mossy-parallel fiber system in the regulation of cerebellar cGMP content. Harmaline, a drug that stimulates climbing fiber activity increases the cerebellar cortex cGMP content but fails to change the cGMP content in deep cerebellar nuclei. In contrast, isoniazid, a drug that decreass GABA levels in cerebellum increases the cGMP content in cerebella cortex and deep cerebellar nuclei. Therefore, these results suggest that harmaline interferes with a set of cerebellar regula tory mechanisms for cGMP which differs from that activated by isoniazid. Such inferences are supported by the results of the experiments with 3-acetylpyridine (3AP). In rats, this neurotoxic agent causes a complete destruction of the inferior oli nucleus neurons and a concomitant degeneration of the cerebell climbing fibers. When harmaline is injected 4 or 30 days afte 3AP, the tremor is abolished and the increase of cerebellar co cGMP is abated. On the other hand, the increase of cerebellar cGMP induced by isoniazid remains unchanged in rats pretreated with 3AP. Thus, these experiments contribute biochemical evid supporting electrophysiological studies showing that the tremo

by harmaline is mediated through an activation of the olivo-cerebellar system. The present results strengthen our previous suggestion that the exitatory transmitter released from the climbing fibers activates a guanylate cyclase system in the Purkinje cells. Glutamate, a cerebellar putative neurotransmitter that increases the discharge rate of Purkinje neurons when applied electrophoretically is equipotent in raising the cGMP content in cerebellum of normal and 3AP treated rats. Evidently, the transmitter released by the endings of parallel fibers increases the cGMP content of cerebellum by the same extent in control and 3AP treated rats. This suggests that the mossy-parallel fiber system is not affected by 3AP treatment and that isoniazid is increasing cGMP through an activation of this system. Our experiments with 3AP and isoniazid also suggest that the putative transmitter released from the parallel fibers probably stimulates guanylate cyclase. The use of 3AP as a chemical tool and cGMP as a biochemical marker of the postsynaptic receptor function, allows us to characterize mechanisms and pathways whereby different drugs change cGMP content in cerebellar cortex.

Apomorphine (a dopamine receptor stimulant) increases cerebellar cGMP content. Haloperidol (a dopamine receptor blocker), morphine (an opiate receptor agonist) and diazepam (a GABA mimetic compound) all decrease cerebellar cGMP content.

None of these drugs changes the guanylate cyclase activity of cerebellar homogenates, moreover, their action on cerebellar cGMP content persists after 3AP.

Apomorphine increases cerebellar cGMP content when it is injected into the corpus striatum but not when injected into the cerebellum. Thus, the increase of cGMP elicited by apomorphine can be differentiated by that induced by isoniazid because the latter drug increases cerebellar cGMP when injected directly in the cerebellum but not when injected in the striatum. Presumably, apomorphine indirectly activates mossy fibers. Using direct injection in the striatum and cerebellum, the decrease of cerebellar cGMP content elicited by haloperidol and morphine can be differentiated from that elicited by diazepam. Perhaps, haloperidol and morphine reduce the mossy fiber input to the cerebellum.

Considering the important role of the cerebellum in the regulation of the sensory motor cortex function, it is conceivable that studies on the modification of cerebellar cGMP content may become an important tool to study the action of anti-psychotic agents on the correlation between cortex, cerebellum and basal ganglia.

Our proposed course is to define the molecular nature of the messages mediated by the increase of cGMP. We have now established that rat cerebellum contains (1) a large amount of cGMP dependent protein kinase (PK) and (2) high concentrations of a thermostable small molecular protein which inhibits cAMP dependent and cGMP dependent protein kinase.

We intend to study how changes of cerebellar cGMP content can modify the function of the cGMP dependent protein kinase and the interaction of the cGMP protein kinase with the inhibitory protein.

Publications:

Costa, E., Guidotti, A. and Mao, C. C.: A GABA hypothesis for the action of benzodiazepines. In Roberts, E., Chase, T. N. and Tower, D. (Eds.): GABA in Nervous System Function (International Symposium on GABA). New York, Raven Press, 1976, pp. 413-426.

Biggio, G. and Guidotti, A.: Climbing fibers activation in 3',5'-cyclic guanosine monophosphate (cGMP) content in cortex and deep cerebellar nuclei of cerebellum. Brain Res. 28: 258-260, 1976.

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Biggio, G. and Guidotti, A.: Regulation of 3',5'-cyclic guanosin monophosphate (cGMP) in cerebellum by a striatal dopaminergic mechanism. Nature 265: 240-242, 1977.

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Biggio, G., Guidotti, A. and Costa, E.: On the mechanism of the decrease in cerebellar cGMP content elicited by opiate receptor agonists. N. Schmiedeberg. Arch. Pharmacol. 296: 117-121, 1977.

Szmigielski, A., Guidotti, A. and Costa, E.: Endogenous protein kinase inhibitors: Purification, characterization and distribution in different tissues. J. Biol. Chem. 252: 3848-3853, 1977.

Biggio, G., Guidotti, A. and Costa, E.: Regulation of 3',5'-cyclic guanosine monophosphate content in deep cerebellar nuclei Neuroscience, 1977, In press.

Guidotti, A., Biggio, G. and Costa, E.: Action of diazepam, haloperidol, morphine and muscimol on the cGMP content of cerebellum. In Deuiker, P., Raducothomas, C. and Villeneuve, A.(Eds.): Proceedings of the 10th Congress of the Collegium Int. Neuropharmacology. Oxford, England, Pergamon Press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01516-04 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of minor tranquilizers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Amin Suria	Staff Fellow	SMRP NIMH
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	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of benzodiazepines on isoniazid induced convulsions, increase of cerebellar cGMP and anterior pituitary cAMP content was compared to the effect of muscimol and nipecotic acid (powerful GABA mimetic compounds). Benzodiazepines like muscimol and nipecotic acid delayed the onset of convulsions induced by isoniazid in doses that did not protect the animals from picrotoxin or strychnine convulsions. Moreover diazepam and muscimol have a similar order of potency in preventing the increase of cerebellar cGMP or pituitary cAMP induced by isoniazid, picrotoxin, harmaline and reserpine. In the nigro-striatal system benzodiazepines, like other GABA receptor agonists, block the activation of DA neurons induced by antipsychotics.

These data suggest that the pharmacological action of benzodiazepines may be mediated by an effect on GABA neurons. The role of GABA in the action of benzodiazepines is emphasized by experiments showing that diazepam elicits positive cooperativity in the specific binding of GABA to synaptic membranes.

Project Description:

The mode of action of benzodiazepines, which are the most widely prescribed drugs is still unknown. Recent evidence from this laboratory has suggested that benzodiazepines may exert a primary action on GABA mechanisms. The present project intends to further elucidate the mechanisms of action of benzodiazepines:

1) Studies with cerebellum

The cerebellum was used as a model to investigate the action of benzodiazepines because: (a) benzodiazepines injected in large doses produce ataxia which probably reflect a cerebellar action of these drugs; (b) the cerebellum represents a well studied model in which GABA-ergic nerve terminals play a dominant inhibitory role on the excitation of Purkinje cells; and (c) changes of the function of the Purkinje cells can be monitored biochemically measuring the content of cGMP in the cerebellar cortex.

cGMP increases when the afferent excitation to Purkinje cells is depressed. cGMP decreases when the afferent input to cerebellu is decreased or when the activity of cerebellar inhibitory GABA-ergic interneurons is increased. Increases in cGMP content of cerebellar cortex resulting from drug actions on GABA receptors can be differentiated from those due to an action on mossy fiber or parallel fibers by comparing the cGMP content of cerebellar cortex after intracerebellar, intrastriatal and parenteral injections of drugs. The availability of muscimol (3-hydroxy-5-aminomethylisoxazole), a drug resistant to enzymatic degradation in the body and endowed with high intrinsic activity and affinity for GABA receptors, which crosses the blood brain barrier, has facilitated the use of this cerebellar model in pharmacological studies of GABA receptors.

Using this model, we have shown that intraperitoneal diazepam in doses as small as 0.5 mg/kg decreases cerebellar cGMP and antagonizes the increase of cGMP content induced by picrotoxin or isoniazid in cerebellar cortex. These effects were duplicated by muscimol. However, haloperidol (a dopamine receptor blocker), morphine (an opiate receptor agonist) and phenobarbital when injected parenterally also decreased cerebellar cGMP. At first, we could exclude a participation of climbing fibers in promoting the cGMP action of all these drugs because diazepam, muscimol, haloperidol, phenobarbital and morphine also decreased cerebellar cGMP content in animals in which the climbing fibers were destroyed.

Secondly, the intracerebellar injection of diazepam, flunitrazepam and muscimol decreased the cGMP content of cerebellar cortex while haloperidol, morphine and phenobarbital up to 20 times higher than benzodiazepines failed to change cerebellar cGMP.

These experiments indicate that the decrease of cerebellar cGMP induced by intraperitoneal injection of diazepam and muscimol can be due to a direct action of these drugs on the cerebellar synapses. In contrast, haloperidol and morphine decreased cerebellar cGMP with a mechanism that is of extracerebellar origin.

Successive experiments have shown that the decrease of cerebellar cGMP content elicited by parenteral morphine was antagonized by intrastriatal naltrexone, and that elicited by parenteral haloperidol was antagonized by intrastriatal apomorphine. The decrease of cerebellar cGMP induced by parenteral diazepam was inhibited by neither naltrexone nor apomorphine injected intrastriatally. Instead, the increase of cerebellar cGMP induced by intracerebellar injection of isoniazid was blocked by parenteral diazepam and muscimol but not by haloperidol or morphine. Thus the view that diazepam lowers cerebellar cGMP by facilitating GABA transmission directly at the level of dendrites or some of Purkinje cells is upheld by these experiments.

2) Striato-nigral inhibition:

Another biochemical model which can be used to test the GABA mimetic effect of benzodiazepines is the study of the kinetic properties of tyrosine hydroxylase in homogenates of substantia nigra and neo-striatum. GABA neurons projecting from striatum to substantia nigra (SN) establish close synaptic contacts with the dendrites or cell bodies of dopaminergic neurons located in SN. When GABAergic afferents to substantia nigra are inhibited, the firing of DA neurons is increased, the DA turnover rate in neostriatum is also increased and the Km of tyrosine hydroxylase for the pteridine cofactor is decreased.

Based on this anatomical, physiological and biochemical evidence, it was reasoned that diazepam, by facilitating GABA function at the level of substantia nigra, should reduce the DA turnover rate in striatum.

After blockade of striatal DA receptors with haloperidol when presumably the function of the inhibitory GABA neuronal feedback loop was reduced and the firing of DA neurons was increased, the affinity of tyrosine hydroxylase for pteridine cofactor was increased. This effect of haloperidol was potentiated by isoniazid and picrotoxin but was blocked by diazepam or muscimol. As in the case of diazepam, muscimol also increased the intensity of haloperidol induced catalepsy.

3) Hypothalamus - Pituitary

Another brain in which there is a large representative of GABAergic neurons in the hypothalamus. Electrophysiological and bio-

chemical evidences indicate that in hypothalamus decreases of GABA levels or blockade of GABAergic receptors produces an activation of pituitary function. This activation is associated with an increase of cAMP in anterior pituitary. Very small doses of benzodiazepines (50 µg/kg of diazepam) abated the increase of cAMP elicited by blockade of GABAergic function, but failed to block the increase of cAMP induced by reserpine, cold exposure and chlorpromazine.

These results indicate that the action of benzodiazepines may be mediated by an increase availability of GABA at receptor sites. The small doses of diazepam used to obtain these effects are very close to that used in man in order to obtain antianxiety and anticonvulsant effects. To confirm such hypotheses, GABA mimetic compounds were compared to benzodiazepines. In all these experimental situations, muscimol injected in doses (0.2-0.5 mg/kg) produced effects identical to that observed with small doses of diazepam.

Similarly, nipecotic acid, a blocker of GABA reuptake blocked the isoniazid induced increase of anterior pituitary cAMP.

4) Allosteric site for GABA receptor regulation

We have studied whether in presence of benzodiazepines one can observe a specific modification of the specific binding of GABA to postsynaptic receptors. The current evidence indicates that 10^{-6}M benzodiazepines modify the Scatchard plotting of the binding of ^{3}H GABA to synaptic membranes in a position compatible with the view that allosteric site that regulate affinity of receptors for GABA are changed by benzodiazepines.

The benzodiazepines are particularly successful in psychiatry because they reduce anxiety while they produce very little sedation. Several biochemical mechanisms have been suggested to participate in the anticonvulsant and antianxiety effects of benzodiazepines. From time to time, acetylcholine, catecholamines and serotonin have been implicated. More recently it has been proposed that benzodiazepines work on specific "anxiety" receptors. Our data suggested that if such receptors exist they may be associated with GABA receptors where they may specifically bind to an allosteric site. Thus the pharmacological and therapeutic action of benzodiazepines may be mediated by an action of benzodiazepines on GABAergic neurons.

The importance of these observations may go beyond the immediate explanation of the mode of action of benzodiazepines and may represent the beginning of a better understanding of the role of GABAergic neurons in anxiety and in other mental disorders.

We plan to study the molecular mechanism whereby diazepam causes positive cooperativity in GABA binding. Cerebellum will be the area of brain in which most of the studies will be conducted because of the present state of our neuroanatomic knowledge in this tissue. On the basis of the results in this organ, the studies will be extended to other GABA rich areas and to the possible interaction of drugs with allosteric receptors for GABA receptor regulation.

Publications:

Guidotti, A., Biggio, G. and Costa, E.: Action of diazepam, haloperidol, morphine and muscimol on the cGMP content of cerebellum. In Deniker, P., Raduco-Thomas, C. and Villeneuve, A. (Eds): Proceedings of the 10th Congress of the Collegium Internationale Neuropharmacologium. Oxford, England, Pergamon Press, in press, 1977.

Naik, J. R., Guidotti, A. and Costa, E.: Central GABA receptor agonist: Comparison of muscimol and baclofen. Neuropharmacology 15: 479-484, 1976.

Biggio, G., Costa, E. and Guidotti, A.: Pharmacologically induced changes in the 3',5'-cyclic guanosine monophosphate content of rat cerebral cortex: Difference between apomorphine, haloperidol and harmaline. J. Pharmacol. Exp. Ther. 200: 207-215, 1977.

Guidotti, A., Naik, J. R. and Kurosawa, A.: Possible role of gamma aminobutyric acid (GABA) in the regulation of cAMP system in rat anterior pituitary. Psychoneuroendocrinology, in press, 1977.

Guidotti, A.: Methods to evaluate in vivo the activity of GABA receptor agonists. In Deniker, P., Raduco-Thomas, C. and Villeneuve, A. (Eds.): Proceedings of the 10th Congress of Collegium Internationale Neuropsychopharmacologium. Oxford, England, Pergamon Press, in press, 1977.

Guidotti, A.: Synaptic mechanisms in the action of benzodiazepines. In Psychopharmacology: A Generation of Progress. New York, Raven Press, in press, 1977.

Biggio, G., Costa, E., Brodie, B. and Guidotti, A.: Mechanisms by which diazepam, muscimol and other drugs change the content of cGMP in cerebellar cortex. Proc. Nat. Acad. Sci. USA, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01517-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of a high power microwave oven

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief, Section on Neuro-endocrinology	SMRP NIMH
Other:	D. L. Cheney	Chief, Section on Molecular Pharmacodynamics	SMRP NIMH
	J. Meek	Pharmacologist	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Focussed microwave irradiation equipment is described which while not impairing microdissection of rat brain nuclei, spinal cord and other tissues allows the assay of the concentrations of chemicals which undergo rapid metabolism during post-mortem processes.

We are now developing new microwave devices to inactivate enzymes in brain and other tissues of large animals and in free moving animals.

This equipment will offer a unique possibility of measuring the effect of drugs on the steady state and turnover rate of neurotransmitters of metabolites during various stages of sleep.

Project Description:

Transmitter and second messenger content of brain structures undergo rapid postmortem fluctation. To measure concentrations and turnover rates of these molecules, it is necessary to instantaneously inactivate the enzymes that synthesize and metabolize these molecules to minimize postmortem distraction. Microwave radiation has been proposed as a method for killing animals in order to preserve brain structure (Stavinoha et al., Pharmacologist 12: 257, 1970).

A metal wave guide connects the microwave generator (2 KW, 2.45 GHz) to a power window opening. A rat or mouse is placed in an animal holder made of plastic which is mounted under the power window opening with the animal's head exposed to the microwave beam. An electronic system precisely controls the duration of radiation (0.5 sec for mice and 2 sec for rats).

To ascertain that this technique is suitable for measuring concentrations of various constitutents in brain of rats, microwave irradiation was compared with the rapid freeze blowing technique (J. Neurochem. 20: 183-188, 1973). With this procedure, high pressure liquid N_2 is used to blow the brain out of the cranial cavity and freeze it within 0.5 sec. With the procedure that uses microwave radiation, however, it is possible to dissect brain nuclei; dissection is impossible following freeze blowing procedures.

The concentration of a number of endogenous substances in the brain of rats killed by microwave radiation was comparable to that found after freeze-blowing, suggesting that microwave killing was not facilitating the accumulation of brain substrate after death.

Currently, we are attempting to increase the value of microwave radiation in studying putative transmitters in spinal cord and brain of rats by increasing the intensity of the beam. The new microwave source which we are describing will allow a rapid (2-3 sec.) inactivation of the enzyme controlling cAMP and cGMP metabolism in spinal cord and in organs like kidney, spleen, heart etc., without handling the animals. Moreover, we plan also to develop this high power microwave generator that will allow us to focus the microwave on the skull of an unrestrained animal. This technique should allow important progress in the studies of different neurotransmitters and metabolites in spinal cord or brain during various stages of sleep.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01518-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The study of mammalian GABA-ergic mechanisms with gas chromatography-mass spectrometry and stable isotopes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. C. Mao	Senior Staff Fellow	SMRP NIMH
Other:	E. Marco	Visiting Fellow	SMRP NIMH
	A. Revuelta	Visiting Associate	SMRP NIMH
	E. Peralta	Guest Worker	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mass fragmentographic techniques have been used to estimate GABA turnover rates in substantia nigra, N. accumbens, N. caudatus and globus pallidus of rats treated acutely or subacutely with cataleptogenic or non-cataleptogenic antipsychotics. Our results showed that all antipsychotics of either single or repeated injection enhanced GABA turnover rate in N. accumbens. Yet only non-cataleptogenic antipsychotics increased significantly the GABA turnover in substantia nigra and N. caudatus. Thus, our findings suggest that GABAergic neurons in N. accumbens play a functional role in the therapeutic action of antipsychotics and that extrapyramidal symptoms caused by treatment of neuroleptics may be alleviated when the GABAergic activity in the strio-nigral system is enhanced.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective has been to study the GABA turnover in substantia nigra, N. caudatus, globus pallidus and N. accumbens in various pharmacological conditions which mimic clinical situations.

We have used a sensitive gas chromatograph-mass fragmentographic technique developed in our laboratory to estimate GABA metabolic rate in brain nuclei with the use of heavy isotope labeled glucose as a remote precursor of GABA.

Mounting evidence in recent years indicates that GABA is probably the most ubiquitous and important inhibitory neurotransmitter in mammalian brain. For example, GABA has been implicated as the transmitter released by four of the five kinds of neurons existing in the cerebellum. A GABAergic pathway has been identified in the basal ganglia by various experimental approaches. This GABAergic pathway apparently participates in the widely speculated neuronal feedback loop which controls the functional activity of the nigrostriatal dopaminergic route.

In addition, evidence has also been presented to suggest that GABAergic neurons play a functional role in other brain areas such as hippocampus, mesolimbic nuclei, etc.

When rats were acutely treated with classical antipsychotics such as haloperidol, pimozide and chlorpromazine which induce catalepsy in animals and extrapyramidal side effects in patients GABA turnover rates in substantia nigra and N. caudatus were unchanged. On the contrary, clozapine and thioridazine, which induce no catalepsy or extrapyramidal side effects, significantly enhanced GABA turnover in these two nuclei. None of the drugs in the doses we used modified steady-state concentrations of GAB. All the antipsychotics studied, despite their different chemical structures, accelerated GABA turnover rates in globus pallidus and N. accumbens.

Our results with cataleptogenic (e.g., haloperidol, pimozide and chlorpromazine) and non-cataleptogenic antipsychotics on GAB turnover in neostriatum substantia nigra and N. accumbens are of interest in light of the current belief that therapeutic effect of antipsychotics against some symptoms of schizophrenia originates from drug action on mesolimbic dopaminergic mechanisms whereas the extrapyramidal symptoms reflect a disturbance of strio-nigral neuronal operation.

These findings on the action of antipsychotics on GABA turnover suggest that an augmented GABAergic activity in strio-nigral system may lessen extrapyramidal side effects caused by the treatment of the antipsychotics, on the other hand, GABAergic mec

nisms in N. accumbens and globus pallidus is somehow associated with the therapeutic efficacy of antipsychotics. The observation that another antipsychotic, (+) butaclamol but not its stereoisomer, (-) butaclamol, also increased GABA turnover in accumbens, further indicates that the change of GABA metabolism in N. accumbens is specific to the drugs with antipsychotic activity.

In order to follow more closely the clinical situation, we have treated animals subacutely with haloperidol and clozapine and studied the changes of GABA turnover. We found that a tolerance was developed to the changes of GABA turnover in globus pallidus after repeated drug treatment but not in N. accumbens. This experiment further strengthens the possibility that GABAergic activity in N. accumbens not that in globus pallidus is involved in antipsychotic action of the drugs.

Currently, we are testing the possibility that the effects of antipsychotics on GABA turnover in brain nuclei, particularly in N. accumbens, are due to their antagonistic action on dopamine receptors.

Publications:

Marco, E., Mao, C. C., Cheney, D. L., Revuelta, A. and Costa, E.: Effects of cataleptogenic and noncataleptogenic antipsychotics on the turnover rate of gamma aminobutyric acid and acetylcholine in rat brain nuclei. Nature 264: 363-365, 1976.

Mao, C. C., Marco, E., Revuelta, A., Bertilsson, L. and Costa, E.: The turnover rate of gamma aminobutyric acid in the nuclei of telencephalon: Implications in the pharmacology of antipsychotics and of a minor tranquilizer. Biol. Psychiatry 12: 359-371, 1977.

Mao, C. C., Cheney, D. L., Marco, E., Revuelta, A. and Costa, E.: Turnover times of gamma-aminobutyric acid and acetylcholine in N. caudatus, N. accumbens, globus pallidus and substantia nigra: Effects of repeated administration of haloperidol. Brain Research, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01519-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Selective activation of neuronal systems by cholera enterotoxin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Neckers	Staff Fellow	SMRC NIMH
Other:	D.-M. Chuang	Staff Fellow	SMRP NIMH
	R. J. Wyatt	Chief, Laboratory of Clinical Psychopharmacology	SMRC NIMH
	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology and Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.95	0.95	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to evaluate the biochemical changes that occur in neuronal systems after the injection of cholera toxin into selected regions of rat brain. Our present studies are directed toward evaluating the consequences of injecting cholera toxin on serotonin metabolism in brain and on evaluating the possible mechanism whereby the toxin activates adenylate cyclase.

PHS-6040
(Rev. 10-76)

Project Description:

Cholera toxin is a non-reversible activator of adenylate cyclase from several tissues. Since adenylate cyclase is thought to be part of the receptor-complex in many neurons, we used cholera toxin to study whether adenylate cyclase was linked to regulatory receptors of serotonergic neurons. The rate of 5-HT metabolism was taken as an index of neuronal activation and we measured this metabolism in brain areas of rats injected with cholera toxin into specific brain regions.

In addition, we have attempted to study the biochemical mechanisms that might come into play when adenylate cyclase is activated by the toxin.

Cholera toxin was injected into the raphe nuclei or caudate nucleus and serotonin metabolism was studied by high pressure liquid chromatography. The biochemical mechanisms for activation of adenylate cyclase, either whole toxin or its protomers, were studied in vitro.

When cholera toxin was injected into the raphe nuclei, the metabolism of serotonin was increased in the striatal regions on both sides of the brain. Increased metabolism was manifested an increase in the concentration of 5-hydroxyindoleacetic acid (5HIAA) for more than 72 hours. The change was a direct result of an increased rate of formation of serotonin rather than an inability of the brain to dispose of the acid. Injection of the toxin or the vehicle for the toxin into the brain ventricles was without effect on the serotonin system. Injection of toxin into the caudate nucleus on one side of the brain resulted in increased levels of 5HIAA in the dorsal raphe nucleus and striatum on the opposite side of the brain. There were no detectable changes of 5HIAA in the medial raphe, whose axons do not project to the striatum. Activation of the dorsal raphe was apparently by a retrograde process. The basal levels of adenylate cyclase were enhanced on the injected side of brain, however, there was no change of adenylate cyclase activity on the contralateral side of brain even though 5HIAA levels increased. The rise of 5HIAA on the contralateral side of brain was prevented by a hemisection caudal to the injection site. Our results suggest that it is possible to biochemically activate the metabolism of brain se[illegible]tc[illegible] for days using regional injections of cholera toxin. Activat[illegible]n is not necessarily associated with long term changes of adenylate cyclase activity. Moreover, it appears that both caudate nuclei may be innervated by a single cell body or by separate cell bodie in ready communication.

The process of adenylate cyclase activation by toxin appears to take place in three stages: a rapid binding of cholera tox[illegible]

to ganglioside GM_1 on the cell surface, a characteristic lag period, and then a rise of cyclase activity. Cholera toxin consists of a subunit A and a subunit B. The B unit is responsible for activating the cyclase. Nicotinamide adenine dinucleotide (NAD) is required for activation. The A unit hydrolyzes NAD. Diphtheria toxin is similar to cholera toxin in that it consists of two units and one of the units hydrolyzes NAD. With diphtheria toxin, the adenosine diphosphate ribose generated by the hydrolysis of NAD is transfered to an acceptor protein which then modifies protein synthesis within cells. We discovered that the hydrolysis of NAD by cholera toxin results in the ribosylation of the A unit itself. Ribosylation was prevented by boiling, low incubation temperature and preincubation with trypsin. The reaction required dithiothreitol and was active in phosphate buffer but not tris buffer. Moreover, the incorporated adenosine diphosphate ribose could not be removed from the A unit by repeated washing with 5% trichloroacetic acid, suggesting that covalent bonding was involved.

Many psychiatric disorders are associated with abnormal metabolism of the biogenic amines in brain. Adenylate cyclase can be activated by the biogenic amines. Cholera toxin offers the opportunity for understanding the mechanisms for activating the cyclases of the amine containing-neurons for prolonged periods.

We will continue our studies of the systems that can be activated by the toxin, and of the basic mechanism that underlie the activation of the neurons.

Publications:

Quenzer, L. F., Galli, C. L. and Neff, N. H.: Activation of the nigrostriatal dopaminergic pathway by injection of cholera enterotoxin into the substantia nigra. Science 195: 78-79, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01520-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The dynamics of catecholamine metabolism in the CNS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. W. Commissiong	Visiting Fellow	SMRP NIMH
Other:	C. L. Galli	Visiting Associate	SMRP NIMH
	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the metabolism of catecholamines in brain and spinal cord and to evaluate the factors that influence amine metabolism. Our present interests are to determine whether dopamine containing neurons are present in the spinal cord and to provide information about their biochemical properties.

PHS-6040
(Rev. 10-76)

Project Description:

Little attention has been given to the possible presence of dopaminergic neurons in the spinal cord because of the lack of sensitive and specific methods for estimating dopamine when it is present in neuronal tissues in low concentrations. Our objecti was to use a gas chromatographic-mass fragmentographic method which is the most specific and sensitive method available to the neurobiologist, to look for dopamine-containing neurons in spinal cord. This dopamine would serve as a transmitter rather than as a precursor for norepinephrine. Drugs that modify dopamine in brain were administered in an attempt to determine if spinal cord dopamine responds to pharmacological agents like brain dopamine.

In the cerebellum, where dopamine serves solely as a precursor for norepinephrine, dopamine represents about 2% of the levels of norepinephrine. In contrast, dopamine represents about 10% of the catecholamines in the cord. The high proporation of dopamine in the cord suggests that it might serve a function other than just as a precursor. In support of a separate dopaminergic system in the cord, we found that pretreatment with desipramine blocked the depletion of norepinephrine, but not dopamine, by the neurotoxin 6-hydroxydopamine. Conversely, pretreatment with benztropine blocked the depletion of dopamine, but not of norepinephrine. Presumably, desipramine and benzotropine prevented the amine reuptake systems in noradrenergic and dopaminergic nerve endings, respectively, form accumulating 6-hydroxydopamine. Moreover, we found that several neuroleptic agents increase homovanillic acid in the cord as they do in the striatum. Circumstantial evidence in the literature suggests that dopamine in the cord might be involved in motor function.

Controversy has surrounded the presence of dopaminergic neuron in the cord, which is based primarily on the lack of specific analytical methods used in the past. We have identified dopaminergic neurons in the cord and have shown that they respond to drugs like the neurons of brain. Abnormal metabolism of dopaminergic neurons in brain has been implicated in several psychiatric and neurologic disorders. Our studies suggest that abnormal dopamine metabolism could extend to the cord and be involved in much of the abnormal motor movements associated with psychiatric and neurologic disorders as well as with some of the side effects to drugs.

In the future, we will attempt to identify motor actions that are modulated by spinal cord dopaminergic neurons. In addition, we will attempt to define the dopaminergic system in cord by fluorscent histochemistry.

Publications:

Karoum, F., Neff, N. H. and Wyatt, R. J.: Distribution and turnover rate of vanillylmandelic-acid and 3-methoxy-4-hydroxy-phenylglycol in rat brain. J. Neurochem. 27: 33-35, 1976.

Karoum, F., Neff, N. H. and Wyatt, R. J.: The dynamics of dopamine metabolism in various regions of rat brain. Europ. J. Pharmacol., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01521-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Functional role of substance P in brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Hong	Visiting Fellow	SMRP NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
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NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study on distribution of substance P in brain indicated that this peptide is most concentrated in substantia nigra and interpeduncular nucleus. By lesion studies, the substance P containing cells bodies which innervate substantia nigra and interpeduncular nucleus were identified to be located in striatum and habenulla, respectively. The chronic treatments with haloperidol lead to a 50% decrease in substance P concentration in substantia nigra. This action of haloperidol appears to be expressed exclusively in the substance P content of substantia nigra. The result suggests a possible interaction between dopaminergic neurons and substance P containing neurons in substantia nigra.

PHS-6040
(Rev. 10-76)

Project Description:

It has been suggested that substance P may serve as a neurotransmitter or neuromodulator in brain. In order to increase our understanding of the physiological role of substance P in central nervous system, we have studied 1) the substance P containing neuronal pathway and 2) the effect of dopaminergic activity on the substance P levels in different regions of rat brain.

A specific antiserum to substance P was produced in rabbits, with this antiserum, a sensitive radioimmunoassay for substance P was developed.

The study of regional distribution of substance P revealed that this peptide is most concentrated in substantia nigra and interpeduncular nucleus. We have examined the location of substance P containing cell bodies which innervate these two areas. The substance P contained in interpeduncular nucleus is stored in nerve terminals originating from habenula. The substance P containing nerve terminals of substantia nigra originate mainly from striatum. Since substance P containing neurons of the striato-nigral projection may regulate DA neurons by a feedback excitation, the more precise location and possible function of this pathway were further examined. Through the use of electrolytic, chemical and mechanical lesions, it was found that substance P containing neurons and GABA neurons course from striatum to substantia nigra in a parallel fashion. However, evidence was provided to indicate that substance P and GABA reside in different neurons. Since it was reported that substance P has excitatory and GABA has inhibitory effect on nigral dopaminergic neurons, the elucidation of this pathway suggest that GABA and substance P may have exert a reciprocal antagonism on the excitability of dopaminergic neurons of substantia nigra. To further analyze the possibility of an interaction between substance P containing neurons and dopaminergic neurons, the effects of chronic administration of antipsychotic drugs on the concentration of substance P in certain brain regions was studied. Acute administration of haloperidol (1 mg/kg) did not alter the substance P levels. Following the chronic treatment with haloperidol, there was a 50% reduction of substance P content in substantia nigra. This result suggests that normally there is an inhibitory dopaminergic influence on substance P neurons. Removal of this inhibitory mechanism by haloperidol may increase the release of substance P from nerve terminals which results in the decrease of substance P content in substantia nigra.

We will further explore the mechanism of the effect of haloperidol on the substance P content in substantia nigra.

Publications:

Hong, J. S., Costa, E. and Yang, H.-Y. T.: Effects of habenular lesions on the substance P content of various brain regions. Brain Res. 118: 523-525, 1976.

Hong, J. S., Yang, H.-Y. T., Racagni, G. and Costa, E.: Projections of substance P containing neurons from neostriatum to substantia nigra. Brain Res. 122: 541-544, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01522-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Phosphoprotein phosphatase of pineal gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.-Y. T. Yang	Pharmacologist	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH
	J. S. Hong	Visiting Fellow	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to understand whether phosphoprotein phosphatase participates in the regulation of protein phosphorylation. The phosphoprotein phosphatase is distributed in both soluble and particulate fractions of pineal glands. The properties of particulate bound and soluble phosphoprotein phosphatase are different. Fractionation of the cytosol indicated the existence of two forms of enzymes differing in molecular weight. A thermostable factor which activates the soluble enzyme was demonstrated in pineal glands and furthermore, it was shown to be a protein in nature. Whether the two different forms of phosphoprotein phosphatase and the thermostable activator of the enzyme mediate the regulation of phosphoprotein phosphatase remains to be further investigated.

Project Description:

Phosphoprotein phosphatase catalyzes the release of phosphate from phosphoprotein and is present in several tissues including nerve tissue. Although the existence of an active and an inactive form of phosphoprotein phosphatase indicates the possible regulation of the enzyme, it is not clear whether the phosphoprotein phosphatases participate in the regulation of protein phosphorylation in vivo. In order to understand the role of phosphoprotein phosphatase in regulation of protein phosphorylation, we have studied some basic properties and in vitro regulation of the phosphoprotein phosphatase in pineal glands.

There is a phosphoprotein phosphatase in soluble and insoluble fractions of rat pineal glands. The soluble enzyme is different from that of the insoluble fraction, as only the former is activated by ATP, GTP and $MnCl_2$. It has been reported by other investigators that cAMP dependent protein kinase is also distributed in both soluble and particulate fractions of various tissues. Furthermore, the properties of the soluble and particulate protein kinases are different. Thus, it is very likely that there are two separate systems to regulate protein phosphorylation: one resides in the membrane and the other in cytosol. Each system has a characteristic phosphoprotein phosphatase and protein kinase. Fractionation of the soluble enzyme preparation revealed the existence of two forms of enzyme differing in molecular weight. These two forms can be further differentiated by their sensitivities to $MnCl_2$ and deoxycholate. A thermostable factor which activates the soluble but not the insoluble enzyme was demonstrated in both beef and rat pineal glands. The thermostable factor is protein in nature as it is nondialyzable and trypsin labile. Whether the endogenous activator mediate the regulation of the phosphoprotein phosphatase in pineal glands remains to be an interesting problem for future investigation.

This project has been terminated.

Publications:

Yang, H.-Y. T., Costa, E., Majane, E. A. and Hong, J. S.: Phosphoprotein phosphatase of pineal gland: Some properties of the enzyme and the identification of an endogenous activator. J. Neurochem. 28: 1075-1080, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01523-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of adenylate cyclase activity by the endogenous protein activator

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. E. Gnegy	Staff Fellow	SMRP NIMH
Other:	P. Uzunov	Visiting Scientist	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH
	A. Gallorini	Guest Scientist	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The participation of an endogenous protein activator in the process of adenylate cyclase supersensitivity to endogenous activators was studied. "Chemical denervation" supersensitivity of striatal dopamine receptors was obtained after discontinuation of chronic treatment with antipsychotics. Animals treated with the cataleptogenic antipsychotics, haloperidol and (+)-butaclamol had an amount of the activator in their striatal membranes larger than control rats and the adenylate cyclase activity was more susceptible to DA stimulation than that of control animals. However, rats treated with the non-cataleptogenic clozapine or (-)-butaclamol which is inactive as an antipsychotic had no change in striatal activator content or in the susceptibility of adenylate cyclase to DA stimulation. It is possible that the adenylate cyclase supersensitivity could be accounted for by the increase in the activator protein in the striatal membranes.

Project Description:

Previously we have shown that the endogenous Ca^{++}-binding protein activator, which activates phosphodiesterase and adenylate cyclase, can affect the ability of striatal adenylate cyclase to be stimulated by certain agents. Depletion of activator from striatal membranes by phosphorylation with cAMP dependent protein kinase (Gnegy et al., 1975) causes a marked reduction in the susceptibility of adenylate cyclase to be stimulated by dopamine or by NaF (Gnegy et al., 1976). This led us to postulate that the activator may be operative in causing supersensitivity of striatal receptors for DA. Therefore we studied whether the DA supersensitivity of adenylate cyclase could be due to a greater activator content in striatal membranes.

In rat striata the dopamine receptors can be made supersensitive by "chemical denervation" using chronic injections of certain antipsychotics. We injected rats chronically (21 days) with one of the following drugs: the antipsychotics haloperidol, clozapine and (+)-butaclamol, (-)-butaclamol, the latter is the inactive enantiomer of the antipsychotic (+)-butaclamol. Animals treated with haloperidol or (+)-butaclamol exhibited behavioral supersensitivity to apomorphine from 2-4 weeks following withdrawal of the drug. No behavioral supersensitivity was noted in animals treated with clozapine or (-)-butaclamol. Two weeks after the last chronic injection of rats were sacrificed and their striata were examined for activator content and dopamine-sensitive adenylate cyclase activity.

Animals treated with the cataleptogenic antipsychotics, haloperidol and (+)-butaclamol had 50% greater striatal activator content as compared to saline-treated controls. The increase was associated with an increased susceptibility of adenylate cyclase to be stimulated by DA. The rats treated with clozapine, the non-cataleptogenic antipsychotic, or with (-)-butaclamol, failed to have a change in the activator content and in the susceptibility of adenylate cyclase to DA. Therefore, animals exhibiting behavioral supersensitivity to the dopamine agonist, apomorphine, showed increased dopamine-sensitive adenylate cyclase activity and had elevated levels of activator in the membrane portion of the striata. This indicated that the activator could be very important in the development of supersensitivity.

In order to correlate the increase in activator with the development of supersensitivity a more controlled time course study was performed. Two days following withdrawal of rats from a 10 day haloperidol treatment, supersensitivity of the striatal adenylate cyclase (as evidenced by a lowering of the Ka for dopamine and an increase of activator content in striatal membranes occurred simultaneously. The occurrence of the "in vitro" supers-

tivity of the dopamine-dependent adenylate cyclase was again corroborated by the development of behavioral supersensitivity to apomorphine. These increases lasted for as long as 10 days after the haloperidol treatment.

These studies indicate that the endogenous protein activator could indeed be important in the development of supersensitivity of striatal dopamine receptors. This leads us to postulate that the activator is an integral component of the supramolecular structure of adenylate cyclase in the membrane and could be a link between the regulatory and catalytic subunits of adenylate cyclase.

We are now further examining the association of the activator with adenylate cyclase and its role in different model of supersensitivity.

Publications:

Gnegy, M. E., Costa, E. and Uzunov, P.: The regulation of the transsynaptically elicited increase of 3',5'-cyclic AMP by the endogenous phosphodiesterase activator. Proc. Nat. Acad. Sci. USA 73: 352-355, 1975.

Gnegy, M. E., Nathanson, J. A. and Uzunov, P.: Release of the phosphodiesterase activator by cyclic-AMP-dependent ATP: Protein phosphotransferase from subcellular fractions of rat brain. Biochim. Biophys. Acta 497: 75-85, 1977.

Gnegy, M. E., Uzunov, P. and Costa, E.: Regulation of the dopamine stimulation of striatal adenylate cyclase by an endogenous Ca^{++} binding protein. Proc. Nat. Acad. Sci. USA 73: 3887-3890, 1976.

Gnegy, M. E., Uzunov, P. and Costa, E.: Participation of an endogenous Ca^{++}-binding protein activator in the development of drug-induced supersensitivity of striatal dopamine receptors. J. Pharmacol. Exp. Ther., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01524-02 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Modulation of Dopamine Metabolism in the Sympathetic Ganglion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Lutold	Guest Worker	SMRP, NIMH
Other:	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP, NIMH
	F. Karoum	Staff Fellow	SMRC, NIMH
	R. J. Wyatt	Chief, Laboratory of Clinical Psychopharmacology	SMRC, NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine is present in a special type of cell in sympathetic ganglia. The cells are known as small intensely fluorescent cells (SIF cells) because of their appearance after histochemical staining for catecholamines. These cells probably modulate information transfer from preganglionic neurons to postganglionic neurons. Our present objective was to evaluate the influence of the preganglionic neurons on the metabolism of dopamine in several sympathetic ganglia of the rat.

Project Description:

Dopamine released from special cells in sympathetic ganglia, known as SIF cells, may function as a transmitter or modulator for slow inhibitory post-synaptic potentials. Our objective was to evaluate the influence of preganglionic neurons on the metabolism of dopamine in rat ganglia. This was accomplished by contrasting dopamine metabolism in normal and surgically decentralized ganglia. In addition, cholinergic drugs were evaluated for their effects on dopamine metabolism.

Dopamine and its metabolites were assayed in the rat superior cervical, middle-inferior cervical and celiac ganglia by mass fragmentography.

In the rat superior cervical, middle-inferior cervical and celiac ganglion 3,4-dihydroxyphenylacetic acid (DOPAC) is the major metabolite of dopamine. The concentration of DOPAC in the ganglia is directly related to the rate of formation of dopamine. Using the superior cervical ganglion as a model we observed that treatment with carbachol increased DOPAC in a decentralized ganglion but not in a sham surgery ganglion. Treatment with atropine followed by carbachol resulted in a decrease of DOPAC in both ganglion, while treatment with hexamethonium followed by carbachol resulted in an increase of DOPAC in both ganglion. Apparently stimulation of muscarinic receptors increases the conversion of dopamine to DOPAC and stimulation of nicotinic receptors decreases the conversion of DM to DOPAC. The increase of DOPAC in the decentralized ganglion following carbachol treatment may indicate super-sensitivity of cholinergic receptors. Based on both dose- and time-response studies with carbachol, the celiac ganglion appears to be the most sensitive of the ganglion studied to carbachol administration.

Neuroleptic drugs are assumed to be effective in the treatment of some psychiatric disorders because they alter dopaminergic systems. The rat ganglia may be a simple mammalian system for evaluating the pharmacology of these drugs as well as other drugs. Our present studies are providing the basis for future studies.

One of the side effects of treatment with neuroleptic drugs is hypotension. We will evaluate the response of the celiac ganglion, which plays a prime role in blood pressure regulation, to the administration of neuroleptic drugs.

Publication:

Karoum, F., Garrison, C. K., Neff, N. H. and Wyatt, R. J.: Transsynaptic modulation of dopamine metabolism in the rat superior cervical ganglion. J. Pharmacol. Exp. Ther., In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01525-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Regulation of nerve growth factor content of nervous system-derived tissues.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Schwartz	Senior Staff Fellow	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nerve growth factor (NGF) is synthesized and secreted by two cell lines derived from the nervous system, the rat C6 glioma and the mouse NB2a neuroblastoma. The content of NGF is increased in either cell line by agents which elevate cyclic AMP: agonists of β-adrenergic receptors for the glioma cells and prostaglandin E_1 or adenosine for the neuroblastoma cells. Research is being carried out to determine: 1) whether the increase in NGF content caused by stimulation of specific receptors causing an increase of cAMP content is the result of new synthesis or of activation of a NGF precursor and 2) whether comparable changes of NGF content can be shown in sympathetic ganglia following administration of β-receptors by injection or release of β-adrenergic receptor agonists.

PHS-6040
(Rev. 10-76)

Project Description:

This project was initiated to determine whether nerve growth factor (NGF) was synthesized by either of the two nervous systems derived cells lines, the rat C6 glioma and the mouse NB2a neuroblastoma, and how the content of NGF was regulated in these cell The cells are grown in monolayer cultures using standard tissue culture procedures. NGF has been measured by two different assay a radioimmunoassay utilizing ^{125}I-β-NGF and antibody to NGF and a bioassay which measures the outgrowth of neurites from dorsal roo ganglia removed from 9-10 day chick embryos. The two assays have given comparable results in all the experiments.

The C6 glioma cells synthesize and secrete NGF. The content of NGF can be increased by treating the cells with beta-adrenergic receptor agonists which activate adenylate cyclase, thereby causing an increase in the intracellular cyclic AMP which is maximal within 15 minutes. The cellular content of NGF increases within hours and the medium content increases in parallel. The response is specific for beta-receptor atonists: alpha-adrenergic receptor agonists are ineffective and only beta-adrenergic antagonists can block either the increase of cyclic AMP or that of NGF. A series of other putative neurotransmitters were ineffective in altering the content of either cyclic AMP or NGF.

These results suggest a possible mechanism of glial-neuronal interaction. Glial cells located in the vicinity of noradrenergic axon terminals may be responsible for supplying these neuron with NGF. During a protracted increase of neuronal activity, an excess of norepinephrine (NE) may accumulate extraneuronally as the reuptake process becomes saturated in the presence of an exc of released NE. This NE that spills over from the synaptic gap may stimulate specific glial cell beta-adrenergic receptors and cause an increase in glial cyclic AMP. This increase could ther result in an increased production and secretion of NGF which would interact with the neurons either by stimulating specific membrane receptors or by being transported via retrograde flow t the neuronal cell bodies. In the cell bodies NGF could increase the rate of production of materials important for the maintenanc of sustained rates of neuronal activity.

The NB2a neuroblastoma cell line also synthesizes and secret NGF. This cell line has receptors for prostaglandin E_1 and fo adenosine but not for catecholamines. Either prostaglandin E_1 adenosine treatment causes an increase of cyclic AMP followed within hours by an increase of NGF both within the cells and in the medium. Other transmitters which are ineffective in elevat of cyclic AMP in these cells also do not change the NGF content These results thus further substantiate the concept that a rise in intracellular cyclic AMP mediates the stimulation of NGF con tent, presumably via an activation of protein kinase.

This research is being carried further in two main directions. We are interested first in determining whether the increase in NGF content seen in the two cell lines is the result of increased synthesis or of activation of a precursor. Preliminary experiments suggest that new synthesis is not involved. Experiments are underway to determine whether the effects of cyclic AMP are on a peptidase thought to be responsible for the conversion of an inactive precursor to the active form.

In order to test our model for glial-neuron interaction, we are looking at regulation of the NGF content of superior cervical ganglia and of various brain regions to determine whether circulating catecholamines or exogenously added catecholamines can affect the amount of NGF in these areas.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01526-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Modulation of tyrosine hydroxylase activity in retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Iuvone	Guest Worker	SMRP NIMH
Other:	C. L. Galli	Visiting Associate	SMRP NIMH
	N. H. Neff	Chief, Section on Biochemical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the physiological mechanisms that are responsible for modulating tyrosine hydroxylase activity in the retina. This enzyme is apparently rate-limiting for catecholamine biosynthesis. Our present interest is to determine whether tyrosine hydroxylase activity of retina is modulated by light.

Project Description:

Tyrosine hydroxylase is rate-limiting for catecholamine biosynthesis. Work from this laboratory has shown that brain tyrosine hydroxylase exists in two forms - a basal and activat state. Activation is manifested by an increase of the affinit of the enzyme for a pteridine cofactor resulting in an increase of enzyme activity in the presence of subsaturating concentrations of cofactor. The assumption is that there is a direct correlation between neuronal firing rates and activation of the enzyme. The amacrine cells of retina contain dopamine. These cells are thought to modulate impulse flow from the photoreceptors to the optic nerve. Our objective was to determine whether tyrosine hydroxylase of retina exists in the basal or activated state and whether environmental lighting alters the kinetic state of the enzyme. In addition, drugs that our laboratory has identified for their ability to alter the kinetic state of the brain enzyme were evaluated for their ability to alter the kinetic state of the retinal enzyme.

Rats were exposed to constant light or dark for 4 days and the kinetics of tyrosine hydroxylase were evaluated by standard procedures.

Exposure to light resulted in an apparent activation of tyrosine hydroxylase. That is, there is an increased affinity of the enzyme for cofactor in animals from the light environment when compared with animals from the dark environment. There was also an increase of the rate of formation of dopamine in the light-exposed rats when compared with the dark-exposed rats. Moreover, haloperidol, which activates brain enzyme, activated the enzyme of retina from dark, but not light-exposed rats.

Dopamine transmission may be impaired in schizophrenia. This notion developed because many drugs that are used to treat this disorder modify the biochemistry of dopaminergic neurons of brain. For example, they block dopamine receptors which results in a compensatory increase of neuronal firing rates and activation of tyrosine hydroxylase. The retinal dopaminergic system offers a simple model for evaluating the biochemical processes that are responsible for the physiological activation of tyrosine hydroxylase. Only by understanding normal physiological mechanisms will it be possible to understand pathophysiology and the mechanism of action of drugs used to treat diseases

In the future, the mechanism for the activation of the enzyme by light and drugs will be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01527-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of endogenous brain opioids using high pressure liquid chromatography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Bohan	Staff Fellow, PRAT Program	SMRP NIMH
Other:	J. L. Meek	Pharmacologist	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method was developed to separate met and leu enkephalin from each other and from the closely related peptides which are their metabolites or potential precursors. This methodology permitted (1) studies of the catabolism of labelled enkephalin in vitro and in vivo and (2) isolation of endogenous enkephalin from rat striatum for quantitative assay. Studies are under way of the formation of enkephalin using labelled precursors.

PHS-6040
(Rev. 10-76)

Project Description:

The discovery of Snyder, Terenius and others of endogenous brain receptors for opiates and the discovery by Hughes et al. of the peptide nature of two of these (met and leu enkephalin) have essentially given birth to a new era in exploration of brain chemistry and its relationship to mental disorders. Among the first steps to be required for exploitation of these discoveries is the development of methodology for measurement of the concentration of the enkephalins in tissues, the elucidation of the biosynthetic origin of the compounds, and establishment of the mechanisms of their degradation. The objective of this project is to attack these problems using as a tool the tremendous capability for purification possible with high pressure liquid chromatography (HPLC).

This method involves adding a tissue extract to a stream of buffer pumped at high pressure (500 psi) over a chromatographic column. Our initial experiments showed that it was possible to separate in less than 5 min both of the peptides of interest (met and leu enkephalin) from each other and from their longer and shorter homologs.

We first utilized this technique to study enkephalin catabolism in vitro and in vivo. We incubated labelled enkephalin with rat brain tissue, or injected it directly into the brain, then separated the enkephalin from its metabolites by HPLC. We found that:

1. Enkephalin is metabolized extremely rapidly by an amino peptidase.

2. This enzyme can be inhibited by o-phenanthroline.

3. The enzyme is uniformly distributed in the brain, unlike enkephalin itself.

4. When enkephalin is injected intraventricularly, more than 9[illegible] is broken down within 1 min.

We next used HPLC to permit a high degree of tissue purification in the assay of met enkephalin in rat corpous striatum. Dr. Yang in this laboratory (Z01 MH 01530-01 SMRP) has been able to measure the met enkephalin concentration in this tissue, but that work required verification due to the variety of other compounds which might cross react in such a procedure. Our separation procedure by HPLC is quite different and depends on a physical separation of the enkephalin from interfering material. Since we obtained a similar value to that of Dr. Yang, despite totally

different methods, the true value is probably close to these.

There are several conclusions of significance in this work: first, the tremendous speed of degradation of enkephalin by membrane preparations and whole tissue that we noted shows that caution is required in interpreting data from previous workers who did not take care to inhibit enkephalin breakdown, and second, the enkephalin concentration determined in tissue by this work and that of Dr. Yang is more than 10 times greater than that obtained by earlier workers. It is likely that this difference is due to our efforts to maximize recovery, and minimize the degradation of enkephalin by tissue. Correct measurement of the enkephalin concentration is essential if we are to understand factors which affect enkephalin synthesis and utilization. The main practical usefulness of our work is to provide a rapid easily used technique for the isolation of peptides. Work now in progress in our laboratory aims at elucidating the biosynthetic pathway for enkephalin synthesis in tissues.

We will attempt to 1) determine the enkephalin concentration of a tissue which can be maintained in good condition in vitro (the intestine); 2) determine whether enkephalin concentration changes after protein synthesis is inhibited to establish whether enkephalin can be synthesized from preformed proteins; 3) incubate the tissue with labelled amino acids and look for the synthesis of labelled precursors (beta endorphin) and labelled enkephalin.

Publications:

Meek, J. L., Yang, H.-Y. T. and Costa, E.: Enkephalin catabolism in vitro and in vivo. Neuropharmacology 16: 151-154, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01528-01 SMRP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Characterization of the neurochemicals involved in the carotid body chemoreceptor mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Hellstrom	Visiting Fellow	SMRP NIMH
Other:	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

I. Hanbauer, NHLI

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have characterized the involvement of catecholamines and other putative neurotransmitters in the carotid body chemoreceptor mechanisms, mainly during conditions of hypoxia. Current studies suggest that cholinergic and hormonal control may modulate a physiological role played by dopamine and norepinephrine in chemoreceptor excitability.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study catecholamines (CA) and other putative neurotransmitters in the regulation of the chemoreceptor function of carotid body. The biochemical assays were performed with mass fragmentography.

CA and Hypoxia: We have previously shown (Brain Res. 118: 352-355, 1976) that the carotid body dopamine (DA) content is decreased during hypoxia while that of norepinephrine (NE) is unchanged. In our current studies we have aimed at determining the mechanisms that regulate DA content of carotid body and to clarify whether hypoxia causes inhibition of synthesis or increase in the rate of release without correspondent increase in synthesis.

I. We could demonstrate that hypoxia (keeping rats ventilating 5% oxygen for different periods of time) did not affect the catecholamine content of other tissues; adrenal medulla, superio cervical ganglion (SCG) and caudate nucleus. The degree of depletion of carotid body DA was related to the concentration of 0_2 in the ventilation gas mixture and duration of hypoxia.

II. Transection of the carotid sinus nerve (CSN), transection the preganglionic sympathetic nerves, removal of SCG or a combination of CSN transection and SCG removal did not significantl change the level of carotid body DA. None of these surgical prc cedures prevented or reversed the DA depletion occurring during hypoxic conditions.

Some changes of the NE content were observed in these denei vation experiments. Thus, SCG removal decreased the content of NE (50%) and transection of CSN caused an increased level of carotid body NE. The former finding would mean that at least ha of the carotid body NE is stored in nerve fibers and terminals originating from the SCG. We have no interpretation of the lat finding but this observation will be an object for future inves gation.

III. The synthesis of carotid body DA was studied by measuring the elimination rate of the metabolites of DA - HVA and DOPAC. DOPAC was found to be the major metabolite. The elimination of DOPAC was studied by inhibiting (with pargyline) the MAO for different periods of time at different times following the onse of hypoxia while DA was kept at steady state.

The elimination rate of DOPAC from carotid body during roo air conditions was found to be fast (T 1/2 of a few minutes). However, the DOPAC concentration is much smaller than that of though the calculated turnover rate of DA is only slightly fa

than that of other neural stores of this transmitter. Since the turnover was unchanged during hypoxia, we have inferred that hypoxia releases DA without changing the rate of synthesis.

Effects of cholinergic drugs on carotid body CA:

Metacholine, a specific muscarinic receptor agonist, known to strongly increase the chemoreceptor fiber discharge, was shown to cause a depletion of carotid body DA. The content of NE was mainly unaffected. Metacholine failed to cause the decrease of carotid body DA in CSN transected animals. In intact animals the depletion of DA could be prevented by injections of atropine, an anticholinergic drug acting on muscarinic receptor sites.

Effects of steroid hormones on carotid body CA:

We showed earlier (Acta Phys. Scand. 93: 540-547, 1975) that chronic treatment with dexamethasone increased by many-fold the amount of carotid body DA as well as NE. Current studies showed that a single dose of dexamethasone, increased by two-fold the DA level. The maximal increase was observed about 12 hrs after the injection. The time course of the NE increase is slower than that of DA. Dexamethasone affected the carotid body CA in a dose-dependent manner. Other steroids, e.g., mineral corticoids did not affect the carotid body levels of DA.

The elimination rate of DOPAC during glucocorticoid treatment was significantly reduced. Protein synthesis blocking agents (actinomycin D and cycloheximide) failed to prevent the increase of the CA. These results show that the increase is not due to an increased synthesis of DA. More probable, the increase of DA might be related to a decreased degradation caused by an inhibition of MAO.

Our findings indicate that DA is released from its stores, the carotid body type I cells, during hypoxia. The effect of hypoxia may act either directly on these cells or on the cholinergic neurons that control the activity of type I cells. The DA released from these cells may modulate the chemosensory discharge. The cholinergic component may be directly involved with the chemosensory receptor events.

Finally circulating levels of glucorticoids may take part in regulating the levels of carotid body CA levels thereby assuming a wider range of adaptability in the DA regulation.

Further studies will be done to expand our knowledge of the role of different neurochemicals, mainly CA, in carotid body mechanisms. The approach will be the same, pharmacological, using

predominantly biochemical techniques but also morphological (including immunohistochemistry) methods at both light and electron microscopical levels.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01529-01 SMRP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Possible role of angiotensin as a neurotransmitter in central nervous system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Fratta	Visiting Fellow	SMRP NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP NIMH
	J. S. Hong	Guest Scientist	SMRP NIMH
	E. Costa	Chief, Laboratory of Pre-clinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the role of angiotensin in central nervous system, angiotensin I converting enzyme which was highly localized in striatum, was studied. Our results revealed the neuronal localization of the enzyme in the striatum. The possible neurotransmitter role of angiotensin in central nervous system will be further explored by studying the regional distribution and regulation of angiotensin in brain.

PHS-6040

Project Description:

Nerve terminals containing specific angiotensin II immunofluorescence were found in several brain nuclei and in the spina cord. Because of the neuronal localization of the converting enzyme in various brain areas, it was suggested that angiotensin II or an angiotensin II like peptide may serve as a putative neurotransmitter.

Angiotensin I converting enzyme was assayed by measuring the His-Leu released from the substrate, Hip-His-Leu fluorometrical The angiotensin was measured by radioimmunoassay.

Angiotensin I converting enzyme was highly concentrated in striatum. Hemitransections at 438 μM led to a 50% reduction in the enzyme activity in the striatum but not in the substantia nigra. Furthermore, by subcellular fractionation, it was revea ed that most of the mitochondrial angiotensin converting enzyme activity is localized in the synaptosome containing fraction. However electrolytic lesion of substantia nigra failed to chang the level of the angiotensin converting enzyme activity in striatum. These results suggest that striatum contains axons which store angiotensin converting enzyme, the cell bodies for these axons are located somewhere in the lower part of the b but probably they are not located in substantia nigra. By radi immunoassay, angiotensin I like peptide was detected in the hypothalamus of rats killed with microwave irradiation.

We will attempt to study the regional distribution and regulation of angiotensin II in CNS.

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PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regional distribution and regulation of enkephalin in brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.-Y. T. Yang	Pharmacologist	SMRP NIMH
Other:	J. S. Hong	Guest Scientist	SMRP NIMH
	W. Fratta	Visiting Fellow	SMRP NIMH
	E. Costa	Chief, Laboratory of Preclinical Pharmacology	SMRP NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is to understand the physiological role of enkephalins in brain. Enkephalins were found to be unevenly distributed in the brain with the highest concentrations in globus pallidus and caudate and lowest in hippocampus and cortex. Furthermore, it was demonstrated that cell bodies of striatal enkephalin may be located in neostriatum or globus pallidus. Chronic treatments with haloperidol, led to a marked increase in striatal enkephalin content suggesting the possible involvement of striatal enkephalinergic neurons in the action of this antischizophrenic drug.

PHS-6040
(Rev. 10-76)

Project Description:

Enkephalin, an endogenous ligand for opiate receptor, has been isolated and characterized. Although pharmacological properties of enkephalins resembles that of opiate alkaloids, their physiological role still remains obscure. In order to explore the biological function and possible involvement of enkephalins in opiate tolerance and physical dependence, we have studied the regional distribution and regulation of enkephalin in brain.

For the measurement of enkephalins, radioimmunoassays for met- and leu-enkephalins were developed. Regional distribution of enkephalin was determined in rats killed by focused microwave irradiation.

There was more than 100-fold variation in the concentration of enkephalin in 34 brain regions studied with the highest concentrations in globus pallidus and caudate and lowest in cerebellum and hippocampus. Furthermore, lesion studies have revealed that neostriatum may contain a considerable number of met-enkephalin interneurons which are intrinsic to the striatum perhaps localized in globus pallidus.

It had been reported that the content of endogenous ligand for opiate receptors is increased in spinal fluid of schizophrenics In order to evaluate the interest of enkephalin as a substrate for the action of antischizophrenic drugs, the brain content of enkephalin was measured after acute and chronic haloperidol. This drug given for 1 week or longer enhances selectively enkephalin content in striatum. To evaluate the latitude of this finding we studied the action of pimozide, clozapine, chloropromazine on enkephalin level in striatum. Also, chronic treatments with pimozide or chlorpromazine led to a marked increase in striatal enkephalin levels like haloperidol, also these two drugs failed to alter enkephalin content in other brain regions. Enkephalin was shown to exert morphine-like activities in vitro and in vivo, however, chronic morphine treatment and pain stimulation by electrical shock were shown to have no effect on steady state levels of enkephalin in our study.

Because of the possible involvement of endorphins in psychotic disorders, we have examined the existance of enkephalin in CSF. A very low level of enkephalin-like material was detected in monkey CSF with the antiserum against met-enkephalin. This endogenous ligand is not structurally homogenous; two species of material binds to antiserum directed toward enkephalin. This material is currently being characterized.

The results of this study indicated that enkephalin is a putative neurotransmitter which is present in and is involved in physiological regulation of functions other than basic detection. The profound effect of haloperidol on enkephalin content of striatum suggests that enkephalinergic activity may be involved in the mediation of the antischizophrenic action of antischizophrenics.

We will attempt to (1) investigate the mechanism whereby haloperidol increases enkephalin content, (2) characterize the CSF enkephalin-like material and (3) study the effects of various drugs and brain stimulation on enkephalin turnover.

Publications:

Yang, H.-Y. T., Hong, J. S. and Costa, E.: Regional distribution of leu- and met-enkephalin in rat brain. Neuropharmacology 16: 303-397, 1977.

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